Appendix 2B-5: Evaluation of the Effect of Surface Water, Pore Water, and Sediment Quality on the Everglades Mercury Cycle

Larry Fink

KEY FINDINGS AND OVERALL ASSESSMENT

Along the well-studied "F" transect in the nutrient-impacted area of WCA-2A, there is a roughly 10-fold increase in the average mosquitofish mercury concentration from the most phosphorus-impacted site, F1, to the unimpacted site, U3. There is a roughly 10-fold decrease in the average surface water phosphorus concentration between those same sites. It has been inferred that the latter is causing the former through a loss of biodilution. In lakes, biodilution is mediated primarily by floating, one-celled plants (e.g., phytoplankton), mats of one-celled plants (e.g., periphyton), and multi-celled plants (e.g., water lilies) that take up Hg(II) (Hg(II)), and MeHg (MeHg), primarily from the water column. In shallow lakes and wetlands, rooted plants take up these mercury species from both the water and sediment, with the relative contributions from each being highly species dependent. Along the "F" Transect, if biodilution is occurring, it cannot be mediated by floating plants, because where the biodilution effect is supposed to be at a maximum at F1, shading by the dense cattail canopy has virtually eliminated floating plants. Conversely, at U3, where the biodilution effect is expected to be minimal, greater light penetration actually increases the biodilution of Hg(II) and MeHg associated with floating plants. If rooted plants must be included in the biodilution calculations, then the ten-fold greater ability of cattail to bioconcentrate MeHg from sediment at F1 than sawgrass at U3 further weakens the evidence for biodilution as the cause. Thus, it is highly unlikely that the apparent strong inverse relationship between water column total phosphorus and mercury bioaccumulation in mosquitofish is due to a loss of biodilution, so some other factor or set of factors must be influencing the bioaccumulation of MeHg in mosquitofish. This probably explains why the apparent strong inverse relationship with surface water phosphorus along the nutrient gradient disappears when sites along that gradient are evaluated individually.

The most likely explanation of the 10-fold increase in mosquitofish mercury levels between F1 and U3 is the approximately four-fold increase in concentration of MeHg in the top 5 cm of soil where MeHg production is believed to be a maximum, magnified by a longer food chain due to the improvement in water quality. There is only a small (< 50%) increase in the concentration of mercury in the sediments between F1 and U3, so that cannot be driving the inferred four-fold increase in the net MeHg production rate. However, there is a roughly three-fold decrease in the average pore water sulfide concentration in surficial sediment (0 - 5 cm). A strong inverse relationship has been observed between the concentration of MeHg on soil solids and the concentration of pore water sulfide in Everglades surficial sediment, and unlike the alleged

inverse relationship with phosphorus, this inverse relationship has been reproduced in the laboratory and in the field under controlled conditions.

Based on nearly seven years of intensive monitoring, research, and modeling, mercury scientists studying the Everglades have concluded that pore water sulfide is likely to be the best predictor of the MeHg production rate in the Everglades, and that the MeHg production rate, not biodilution, is likely to be the best predictor of MeHg bioaccumulation in the Everglades fish. Still, no one-variable empirical model can capture the complexities of the influences of water, pore water, and soil chemistries on the aquatic mercury cycle in the Everglades or elsewhere. Such one-variable models have limited predictive value and are likely to mislead Everglades restoration decision-making by seriously over- or underestimating the magnitude of post-restoration mercury risks.

By contrast, recent modifications to the Everglades Mercury Cycling Model-II (E-MCM(II)) accommodate a number of these complexities, including the effect of phosphorus on MeHg biodilution. This bodes well for the eventual application of E-MCM (II) to the development of effective short-term mitigative measures and long-term operational alternatives to reduce mercury risks arising from the construction and operation of the Everglades Construction Project (ECP) to the maximum practicable extent. Preliminary results of the application of the modified E-MCM(II) to the prediction of post-ECP mercury consequences suggest that there is an ample margin of safety in the District's worst-case analysis of the ecological risks associated with the attainment of the proposed 10 ppb Water Quality Standard for total phosphorus.

Based on the extensive review and analysis contained in this report, there is no need to raise the proposed TP water quality standard of 10 ppb, exempt certain areas from its application, or delay its implementation based on earlier unrealistic estimates of increased mercury risks to fish-eating wildlife attributed to a loss of biodilution. Ultimately, the solution to mercury pollution is not biodilution but source control. The focus of the efforts to understand and correct the Everglades mercury problem should now shift from empirical analysis of monitoring data to controlled laboratory and field studies of the underlying causes of the observed mercury effects. A number of such studies have been completed, are under way, or planned to start in the next fiscal year. The deeper mechanistic understanding of the effect of water, pore water, and sediment quality on the Everglades mercury cycle must then be translated in a realistic way into E-MCM(II), which will eventually be used to develop a mercury Total Maximum Daily Load (TMDL) for the Everglades and derivative emissions reductions.

INTRODUCTION

This report explores the relationship between the physical and chemical characteristics of surface water, sediment pore water, and sediment solids on the concentrations of methylmercury (MeHg) in those media and in fish from the same environments. This report is prompted, in part, by concerns that a change in water quality to be brought about by the construction and operation of Stormwater Treatment Areas is likely to cause of contribute to an exacerbation of the existing, downstream mercury problem in the Everglades. Specifically, this report:

- summarizes literature relevant to the Everglades aquatic mercury cycle within a conceptual modeling framework, including virtually all of the Everglades mercury studies completed to date;

- updates earlier empirical analyses of the relationship between surface water, pore water, and soil chemistries and MeHg (MeHg) bioaccumulation in mosquitofish (*Gambusia holbrookii*) for the well-studied nutrient gradient in Water Conservation Area-2A;

- expands the discussion to include an empirical analyses of the effect of surface water quality on mosquitofish MeHg bioaccumulation in the L-7 Canal at Site ENR 004;

- and mosquitofish, sunfish (*Lepomis sp.*), and largemouth bass (*Micropterus salmoides*) in Water Conservation Areas 1, 2A, 3A, and the Everglades National Park;

- summarizes the results of controlled studies of MeHg bioconcentration and bioaccumulation in laboratory microcosms and field mesocosms;

- reiterates the biodilution calculation carried out previously; and

- presents the preliminary results of mechanistic mathematical modeling of MeHg bioaccumulation along the WCA-2A nutrient gradient under various total phosphorus reduction scenarios.

CONCEPTUAL MODEL OF MERCURY CYCLING IN THE EVERGLADES

HG(II) FATE AND TRANSPORT

Hg(II) is supplied to the Everglades by wet and dry atmospheric deposition, surface flow, and peat soils. Hg(II) then distributes itself amongst the dissolved ($Hg(II)_{aq}$), complexed(L- Hg(II)), and sorbed (S- $Hg(II)_{aq}$) phases in the water column. The Hg(II) can complex with dissolved organic carbon (DOC) (Wallace et al, 1982; Benoit et al., 2001; Haitzer et al., 2002) or sorb to colloids (Wallace et al., 1982; Guentzel et al., 1996; Babiarz et al.,. 2002), bacteria microfilms (Hintelmann et al., 1993), algae and periphyton (D'Itri, 1971; Hakanson, 1980; Hurley et al., 1998; SFWMD 1995-1999; Krabbenhoft et al., 2000; Krabbenhoft and Fink, 2001; Miles et al. 2001; Moye et al., 2002) or floating and rooted macrophytes (SFWMD, 1995-1999; Hurley et al., 1998; Fink and Rawlik, 2000; Krabbenhoft et al., 2000; Riddle et al., 2002). In the Everglades, due to the high concentration of DOC and particles of plant origin (biotic particles), most of the time Hg(II) is in the complexed or sorbed phases, and only a small fraction is in the truly dissolved phase. However, because DOC-complexed Hg(II) will pass through a 0.4 micron filter, one must distinguish between the apparently dissolved (unfiltered minus filtered) and the truly dissolved phases.

Truly dissolved or DOC-complexed Hg(II) can then be transformed (reduced) to dissolved elemental mercury, $Hg(0)_{aq}$ in response to the action of sunlight (Saouter et al., 1995; Amyot et al., 1997), and the reaction generally proceeds faster for the DOC-complexed Hg(II), but in the Everglades neither reaction occurs especially rapidly and both are probably limited to the top few centimeters of the water column, due to the high concentrations of light-absorbing DOC present (Krabbenhoft et al., 1998; Zhang and Lindberg, 2000). Some of the $Hg(0)_{aq}$ produced in this way can be converted (oxidized) back to $Hg(II)_{aq}$ either by direct reaction with dissolved oxidants produced by the action of sunlight on water (Xiao et al., 1994) or on DOC complexes (Xiao et al., 1995; Zhang and Lindberg, 2000). Where the concentration of $Hg(0)_{aq}$ exceeds that required for equilibrium with the concentration in the overlying air, it can also be transferred from water to air

(evasion)(Vandal et al., 1991; Vandal et al., 1995; Lindberg et al, 1999; Lindberg and Zhang, 2000), mediated by temperature and wind speed. At night, when sunlight-driven production ceases, the concentration of Hg(0) in the gas phase in overlying air can exceed that required for equilibrium with the concentration of $Hg(0)_{(aq)}$ remaining in water, and there can be net transfer from the air to water (Lindberg et al., 1999). This process must be distinguished from that which transfers reactive gaseous mercury (RGM) from the air to wet surfaces, whether that of open water or of dew-covered leaves.

Hg(II) reaches the surficial sediment primarily in association with settling particles (Hurley et al., 1994; Watras et al, 1995; Hakanson, 1980; Ambrose and Araujo, 1998), and, all other factors being equal, the Hg(II) settling rate is high where the particle settling rate is high, and vice versa. However, by competing with particles for $Hg(II)_{aq}$, DOC can weaken this link and reduce the magnitude of this proportionality. Movement of dissolved and colloid- or DOC-bound Hg(II) can also occur from the overlying water to the surficial sediment when the concentrations of the former are greater than the latter (Aiken and Reddy, 1997; Reddy et al., 1999). Once deposited to the surficial sediment, the Hg(II) can remain in the form in which it was received or redistribute itself in response to the changing physical, chemical, and microbiological conditions it encounters, the latter being more likely. In the surficial sediment Hg(II) can sorb to or complex with soil particle surfaces, either to the organic fraction (Gilmour et al., 1998b, 1999; Xia et al., 1999) or the iron (or manganese) oxyhydroxide fraction (Yin et al., 1997), be present in soil pore water in true solution, or, more typically, in association with dissolved organic carbon (Ravichadran et al., 1998) or sulfide complexes (Dyrssen, and Wedborg, 1991; Ravichardran et al, 1998; Ravichadran, 1999; Benoit et al, 1999b, 2001). Some of the Hg(II) can be converted by soil microbes to $Hg(0)_{aq}$ (M.Gustin, UNLV, personal communication), then taken up by rooted macrophytes and lost to evasion from leaf surfaces (Lindberg et al., 1999), most likely following the same gas transport pathway as for transpiration (Lindberg et al., 2002).

Neutral or charged complexes of Hg(II) and sulfide ion ($S^{=}$) ($[Hg(II)_xS_y]^{-n}$) form under the appropriate reducing conditions at circumneutral pH, precipitate as HgS(s) (cinnabar). It has also been suggested that mercury-sulfide complexes can co-precipitate with or sorb onto the surfaces of more prevalent iron sulfide complexes ($[Fe(II)_xS_y]^{-n}$) (C. Gilmour, ANSERC, personal communication, 1998). However, processes of precipitation, co-precipitation, or sorption of ($[Hg(II)_xS_y]^{-n}$) can be inhibited by the DOC in pore water (Ravichadran et al., 1998; Ravichadran, 1999). Pore water Hg(II), whether in the truly dissolved or DOC-complexed phase, can be transported back to the overlying water by physically mediated processes (i.e., groundwater exfiltration, dispersion, and diffusion: Thibodeaux et al., 1996; Choi and Harvey, 2000; King, 2000) or biologically mediated processes (i.e., bioturbation, biopumping, or biotransport). Because DOC and inorganic colloids compete with stationary particle surfaces for Hg(II), their high concentrations in pore water facilitate transport out of surficial soils, irrespective of the mechanism. In the Everglades, pore water DOC concentrations generally exceed that in the overlying water column by a factor of two or three (Reddy et al., 1999), so diffusive exchange favors loss to the overlying water. However, this process can be reversed during severe dryout events when the DOC concentration in the overlying waters becomes highly concentrated (Reddy et al., 1999).

Conditions and Factors Influencing Hg(II) Fate and Transport

Based on the above discussion, there are three processes that dominate the fate and transport of Hg(II) in aquatic ecosystems in general and the Everglades in particular. In the most probable order of significance for mass transport and transformation rates in the Everglades, they are

sorption to and settling of organic particles > photochemical reactions = oxidation-reduction reactions > microbial transformation into MeHg. Concomitantly, the conditions and factors that mediate or influence the directions or rates of those processes must have the greatest influence on the fate and transport of Hg(II). The following is a discussion of the influences of dissolved oxygen (DO), total suspended solids (TSS), dissolved organic carbon (DOC), acidity (pH), calcium (Ca) and magnesium (Mg) (individually and expressed as "hardness") on the transport and fate of Hg(II), and total phosphorus (TP).

DO: Dissolved oxygen (DO) is the primary determinant of the rates at which chemical and biological processes requiring oxygen occur in surface waters. However, in the highly organic Everglades sediment, oxygenless (anaerobic or anoxic) conditions exist a few mm down (W. Orem, USGS, personal communication; McCormick et al, 1996), and the electrochemical potential becomes increasingly reducing as one proceeds further down into the sediments for 10 or 20 cm (Reddy et al, 1991), except perhaps in the vicinity of the roots of plants that transport gases via the lacunae (e.g., cattail: Chanton, 1999). Instead, the oxidation-reduction potential in the surficial sediment is dictated primarily by the activity of the various communities of C-, N-, P-, and S-limited anaerobic microbes, moderated predominately by the Fe(II)-Fe(III) and Mn(II)-Mn(III) redox couples, which, in turn, are moderated predominately by pH, $O^{=}$, and $S^{=}$, which are influenced by the activities of the various P-, N-, and S-limited microbes (Reddy et al., 1998a,b,c), closing the biogeochemical loop (Stumm and Morgan, 1996). Water temperature and ionic strength determine DO solubility (Stumm and Morgan, 1996). Sediment temperatures also influence the metabolic rates of the microorganisms that consume and release carbon, nitrogen, oxygen, and sulfur species (Reddy et al, 1998a,b,c; Mark Marvin-DiPasquale et al., 2001).

TSS: In the Everglades, organic and inorganic particles, as measured by total suspended solids (TSS), are supplied from external sources (i.e., stormwater runoff and lake releases directed through District culverts) or an internal source (i.e., plant production and decomposition). The rate of production of plant tissue (biomass) is mediated by some limiting physical, chemical, or biological factor, which for most aquatic ecosystems is light, nitrogen, or phosphorus. In the Everglades, phosphorus is generally limiting, except where the canopy of living and dead cattail stalks, leaves, and stems is so dense that it shades out the other primary producers (Grimshaw et al., 1997; McCormick et al, 1999). Living, dying, and dead particles originating with internal plant production predominate in the waters of the Everglades everywhere but in the areas immediately downstream of District structures, and since phosphorus is the factor limiting the internal production of algae, periphyton, and floating and rooted macrophyte (e.g., water lettuce, water hyacinth), phosphorus must have a significant influence on the transport of Hg(II). Because DOC has a high affinity for Hg(II), its presence in high concentrations can weaken the influence of settling particles on the transport of Hg(II) from the water column to the sediment. The many influences of DOC on Hg(II) biogeochemistry and the many factors mediating those influences are taken up in the next section.

DOC: The quantity and quality of sunlight reaching the Hg(II), whether dissolved or complexed with DOC, is mediated by the quality and quantity of DOC present in surface water (Krabbenhoft et al., 1998). As with particles, this DOC can be supplied by an external or an internal source. The light-absorbing efficiency and affinity for Hg(II) are governed by the quality of DOC and the quality of the DOC is governed by its source. Where DOC concentrations are high, the strong affinity between Hg(II) and DOC will decrease the fraction of Hg(II) in the water column that is associated with organic particles. This will weaken the influence of the net rate of organic particle settling and sediment accumulation on the net deposition velocity of Hg(II). Conversely, factors that weaken the affinity of Hg(II) for DOC more than for organic particles will strengthen the influence of the rate of organic particle settling and sediment accumulation on

the net deposition velocity of Hg(II). The presence of high DOC concentrations in pore water may facilitate the process of transfer of Hg(II) back to the overlying water column by preventing the Hg(II) from strongly sorbing to soil particles or forming sulfide precipitates or co-precipitates (Ravichadran et al, 1998; Ravichadran, 1999).

In the northern Everglades, the quality of the DOC is dominated by the contribution from EAA runoff, which has more aromatic character, while DOC produced from the decomposition of aquatic plant biomass has more aliphatic character. As a consequence of the difference in the quality of the DOC originating with external and internal sources, in the northern Everglades there is a much greater rate of change in the quality of the DOC with downstream distance than in the absolute concentration of DOC. This has implications for the proper analysis of the influence of various surface water constituents on the transport, fate, and bioaccumulation Hg(II) and MeHg in the Everglades and the reliability of predictions based on regression relationships developed from such analyses. Those implications are discussed below and in the section discussing the Conditions and Factors Influencing MeHg Production, Fate, and Transport

It has been observed that the more aromatic DOC that originates with EAA runoff has a higher affinity for Hg(II), while the more aliphatic DOC originating with internal production has less affinity for Hg(II), both of which were contrary to expectations (G. Aiken, USGS, personal communication). This is because the aromatic DOC tends to sorb Hg(II) more by electrostatic interaction, which was thought to be of only secondary importance in this regard. The opposite is generally true for aliphatic DOC, which tends to form coordinate covalent complexes with Hg(II) of varying strengths with (nucleophilic or electron-donating) ligands that decrease in the order sulfhydryl (-SH) >> aminyl (-NH) > hydroxyl (-OH) > arboxyl (-COOH). Since the aromatic DOC has greater affinity for Hg(II) than the aliphatic DOC, either the number of sulfhydryl ligands is much smaller than originally believed, physical access to these sites is blocked by the secondary, tertiary, or quaternary conformations of the molecule, or other strongly binding divalent cations must already be occupying these binding sites. This last explanation is questionable, because Hg(II) has the highest affinity of any soft cation routinely encountered in the aquatic environment, but other soft cations like copper are present in much higher concentrations, so the kinetics may favor Hg(II) binding to weaker binding sites where the exchange rates are much higher. Moreover, the Hg(II) bound to the sulfhydryl groups could be more susceptible to aphotic and photic electron transfer to produce Hg(0) than when bound to the other ligands. Hg(0) is released from the ligand upon formation.

Whatever the cause of the observed effect, the apparent predominance of electrostatic binding of Hg(II) to aromatic DOC requires that factors that weaken electrostatic interactions should have the strongest inverse influence on the magnitude of Hg(II) binding to DOC in the northern Everglades. Such factors include ionic strength, which is proportional to conductivity and the concentration of total dissolved solids, the concentration of hydronium ion (as measured by pH), and the concentration of "hard" cations such as aluminum, calcium, and magnesium, but less so the "soft" cations such as iron, manganese, and copper (Stumm and Morgan, 1996). Conversely, in the waters of the central and southern Everglades, where aliphatic DOC predominates, the opposite relationships should obtain. The complexity introduced by these competing inverse influences, together with the change in the susceptibility of DOC to them as one moves from an area dominated by aromatic DOC in the north to one dominated by aliphatic DOC in the central and south, militates against inferring cause-effect relationships or developing reliable predictive models from empirical observations without corroborating results from controlled laboratory and field experiments.

pH: The negative logarithm of the activity of hydrogen ion in water is the pH. It is proportional to the hydrogen ion concentration. Low pH is associated with a high hydrogen ion activity and represents an acidic environment, while high pH is associated with low hydrogen ion activity in water, with a concomitant increase in the activity of hydroxide ion, which represents a basic environment. In well-buffered aquatic ecosystems, the concentration of hydrogen ion in water govern the equilibrium speciation of inorganic and organic acids and bases that are soluble in water. In poorly buffered systems, the concentration of hydrogen ion is dictated by the concentrations of these inorganic and organic acids dissolved in water. The degree to which an inorganic or organic acid or base contains charged functional groups is dictated by the tendency toward chemical equilibrium and the pH of the system. In addition, all other things being equal, basic conditions favor electron transfer from water to electron acceptor species, while acidic conditions favor electron transfer from the electron donor species to water, so pH also has a direct influence on the rate and direction of important redox reactions. The negatively and positively charged functional groups have affinities for positively charged and negatively charged dissolved ions, so pH mediates the complexation and precipitation of a wide range of anions and cations in aquatic systems. Low pH (acidic conditions) is expected to reduce the affinity of DOC for Hg(II) and MeHg, while circumneutral or basic conditions are expected to increase that affinity.

Hardness: Ca and Mg precipitate as carbonates, with the threshold concentration for precipitation decreasing with increasing pH. Ca and Mg are small, divalent ions, and, as such, interact strongly with negatively charged surfaces and functional groups (Stumm and Morgan, 1996). The association of Ca and Mg with such surfaces and functional groups results in a reduction of the effective charge experienced by other divalent and trivalent cations in solution. Ca and Mg are known to associate with negatively charged colloids, in some cases altering the conformations they assume in solution (Stumm and Morgan, 1996). Because Ca and Mg form stable neutral complexes with a number of organic acids with negatively charged functional groups, Ca and Mg may decrease the affinity of DOC for Hg(II) and MeHg, where the reduced sulfur binding sites on the DOC have already been saturated (Haitzer et al., 2002). By occupying and neutralizing these charged moieties, Ca and Mg may facilitate the reduction in the radius of gyration of these large molecules, causing them to close up and become more colloid-like, further restricting access to the reduced sulfur moieties that would otherwise form the strongest complexes with Hg(II) and MeHg. Binding would then shift from primarily complexation-driven to surface charge-driven, which would further increase the role of pH, Ca, and Mg in mediating Hg(II) sorption to DOC, so low pH would be associated with high DOC colloid concentrations and circumneutral and high pH would be associated with more open DOC molecules and higher concentrations of aluminum and iron colloids.

Phosphorus: Where phosphorus limits primary production and aerobic microbial activity, the production and consumption of DO are both mediated by phosphorus, so phosphorus has an indirect but potentially significant influence on surface water chemistry via the oxygen cycle. This influence can be amplified or diminished by the indirect effect of phosphorus on pH and alkalinity via its direct effect on primary production. Where phosphorous limits plant production or decomposition, it is also a primary determinant of the character and quantity of internally produced (autochthonous) organic particles and dissolved organic carbon (DOC), the flux of settling organic particles from the water column to the sediment and the rate of accretion of undecomposed (refractory) plant biomass that eventually consolidates and compresses and metamorphoses under anaerobic conditions to become peat (W. Orem, USGS, personal communication, 2001).

MEHG PRODUCTION, FATE, AND TRANSPORT

It is known that MeHg is produced where short-chain carboxylic acids are in adequate supply under anaerobic conditions by a variety of natural bacteria (Wood et al., 1968; Jensen and Jernelov, 1969; Olson and Cooper, 1976; Beijer and Jernelov, 1979; Berman and Bartha, 1986; Regnell, 1994; Gilmour et al., 1996; Gilmour et al., 2001) but primarily the sulfate-reducers (Gilmour and Henry, 1991; Henry, 1992; Gilmour et al., 1992; 1998a,b; Benoit et al., 2001). In the Everglades, MeHg production has been observed primarily in the top 4 cm of surficial soil or sediment but not in the water column (Gilmour et al., 1998b; 1999). It has been hypothesized that MeHg is produced from the Hg(II) concentrated at soil surfaces but not so strongly bound that it is unavailable to sulfate-reducing bacteria (Gilmour et al, 1998b; Gilmour et al., 1999). However, defining this bioavailable fraction, either functionally (W. Landing, UF, personal communication) or mechanistically (Benoit et al., 1999a,b; 2001) has proved experimentally challenging. In addition, some MeHg production has been observed in periphyton mats (Cleckner et al., 1999) and the roots of floating macrophytes (Hurley et al, 1999; Guimaraes and Mauro, 1999; Mauro et al., 2000). In the Everglades, this occurs primarily in highly eutrophic, highly sulfidic areas (i.e., WCA-2A-F1; ENR).

Some of the MeHg produced in this fashion is demethylated by a variety of natural bacteria under anaerobic conditions at the sediment/water interface. At high MeHg concentrations, demethylation proceeds by a pathway associated with a detoxification mechanism (Barkay et al, 1996), while at low MeHg concentrations, this pathway is not activated, and demethylation proceeds by various oxidative pathways with the concomitant production of methane or carbon dioxide (Oremland et al., 1991; Marvin-DiPasquale and Oremland, 1998; Pak and Bartha, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001). The remaining MeHg can sorb to soil particles (Yin et al., 1997; Gilmour et al., 1998b; Xia et al., 1999), move into pore water, where it distributes itself between the dissolved and colloid-bound or complexed phase, primarily with dissolved organic carbon (Amirbahman et al., 2002). Form the pore water it can migrate back into the overlying surface water by physically mediated processes (i.e., groundwater exfiltration, dispersion, or diffusion: King, 2000) or biologically mediated by benthic organisms or their predators (bioturbation, biopumping, or biotransport: Krabbenhoft et al., 2001). As with Hg(II), a fraction of the sediment MeHg is so strongly sorbed to particles that it cannot be transferred either to pore water or the microorganisms and macroorgansims living in or on the sediment. The remaining fraction is said to be physically, chemically, and biologically available for reaction, transport, or redistribution to other media. As with Hg(II), MeHg can be transported into the overlying water column by physically and biologically mediated processes. Once present in surface water, MeHg sorbs and settles in a similar fashion to Hg(II) (see above discussion) or is decomposed to Hg(II) or elemental mercury by sunlight (Sellers et al., 1996; Krabbenhoft et al., 1998; D. Krabbenhoft, USGS, personal communication, 2000). Methyl-mercury that sorbs to settling particles can also be demethylated at the soil/water interface.

Factors Influencing MeHg Production, Fate, and Transport

Temperature: All physical, chemical, and biological processes mediated by water have a temperature dependence, while processes driven solely by direct interaction with sunlight do not. The temperature dependence of a reaction generally increases in the order viscosity < diffusion < solubility < chemical reaction $\leq$ biological reaction. However, biological organisms have a preferred temperature range in which they thrive and a tolerable temperature range in which they survive. If either extreme of this tolerable range is exceeded for any length of time, death ensues. The temperature dependencies of methylation and demethylation rates have been measured in

various Everglades soils. Methylation was found to be highly temperature-sensitive (Marvin-DiPasquale et al, 2001) and this sensitivity changes with location, suggesting that some significant change in the physical, chemical, or microbiological environment has occurred between sites. Conversely, demethylation is relatively temperature-insensitive, and this remains fairly constant among all of the sites studied (Marvin-DiPasquale et al, 2001).

Sulfate: The addition of group VI anions to freshwater sediments has been demonstrated to inhibit MeHg production (TeO_4^{-2}- > Se O_4^{-2} > MoO_4^{-2} > W O_4^{-2}: Chen et al., 1997). By contrast, SO_4^{-2} has been observed to stimulate MeHg production at low concentrations but inhibit it at high concentrations (Craig and Bartlett, 1978; Compeau and Bartha, 1984; Berman and Bartha, 1986; Chen et al., 1997; Gilmour et al., 1998b; Benoit, 1999a,b; Jay et al, 2000; Benoit et al., 2001; Marvin-DiPasquale et al., 2001). This is consistent with the laboratory results obtained from dosing Everglades soil cores (Gilmour et al., 1998a,b), soil homogenates (Marvin-DiPasquale et al., 2001), and *in situ* mesocosms (Gilmour et al., 2001). Sulfide is produced by SRB as a byproduct of their metabolism. A strong inverse relationship has been observed between pore water sulfide and the concentration of MeHg in soil (Gilmour et al., 1998b, 1999). This relationship is displayed in **Figure 1**.

DO: Where DO is high, the manifestations of eutrophication are generally absent, so the net MeHg production rate in the surficial sediment is generally lower than in waters with low DO, all other conditions and factors being equal (USEPA, 1997). DO added to a soil microcosm will generally strongly suppress MeHg production (Compeau and Bartha, 1984; Marvin-DiPasquale et al, 2001). However, where sulfate is in high concentrations and DO is in low concentrations, sulfide can build up in pore waters to concentrations that inhibit MeHg production (Choi and Bartha, 1986; Gilmour et al, 1998b; Benoit et al., 1999a,b; Jay et al., 2000; Benoit et al, 2001.) Since sulfate appears to be in excess throughout much of the Everglades, and most methylation occurs in the surficial sediments under highly anaerobic conditions, it is likely that the influence of DO on the rate of MeHg production is muted, while that of sulfate and sulfide is magnified.

Dissolved Organic Carbon: More recently, the stimulation of MeHg production has been observed in a prairie stream in response to the natural addition of organic carbon in the form of decaying leaves (Balogh et al., 2002) and in a mesocosm to which was added DOC concentrated from Everglades waters (D. Krabbenhoft, USGS, personal communication). The former could be the result of the stimulation of microbial activity in conjunction with a DO sag, while the latter is more likely the result of an increase in the bioavailable fraction of Hg(II) in soil due to the inhibition or reversal of sulfide precipitation per the above discussion of the influence of DOC on the Hg(II) cycle. DOC also mediates the disposition of MeHg in the water column, its sedimentation rate, and its physical, chemical, and biological availability for decomposition.

pH: All other conditions and factors being equal, low (acid) pH in poorly buffered lakes and in peat bogs has been associated with the stimulation of net MeHg production. It has been hypothesized that this is brought about by the reduction in the concentrations of inorganic colloids with which it is strongly associated, a reduction in the affinity of the Hg(II) for inorganic and organic colloid surfaces, and/or a reduction in the rate of formation of insoluble carbonate, oxyhydroxide, or sulfide precipitates of Hg(II) or its co-precipitates with other divalent cations (e.g., Ca, Mg, Fe(II), Mn(II)). However, field observations in lakes and wetlands supporting this hypothesis have generally been unable to discriminate the contribution of the sulfate present in acid rain from the effect of pH alone (Gilmour and Henry, 1991).

Ca and Mg: Although the literature provides no clear picture of the influence of Ca and Mg on MeHg production, high DOC concentrations have been demonstrated to slow or inhibit the uptake of nutrients by bacteria by sorbing the exogenous enzymes required for nutrient uptake, and high Ca concentrations have been demonstrated to reverse that effect by reducing the affinity of the DOC for those exogenous enzymes (Wetzel, 1991). Newman et al. (2001) have speculated that the reason that the decomposition of plant tissues proceeds so much more slowly in WCA-1 than WCA-2A is that WCA-1 waters are low hardness, slightly acidic waters, while the waters in WCA-2A are high hardness, slightly basic waters, due to the interaction of surface waters with surficial ground waters that have equilibrated with the limestone carst formations underlying much of the Everglades (SFWMD, 1992). Since DOC has been shown to stimulate MeHg production in an Everglades mesocosm experiment (G. Aiken, USGS, personal communication, 2002), it is possible that high Ca and Mg concentrations could interfere with this stimulation, depending on the mechanism by which DOC exerts its stimulatory effect.

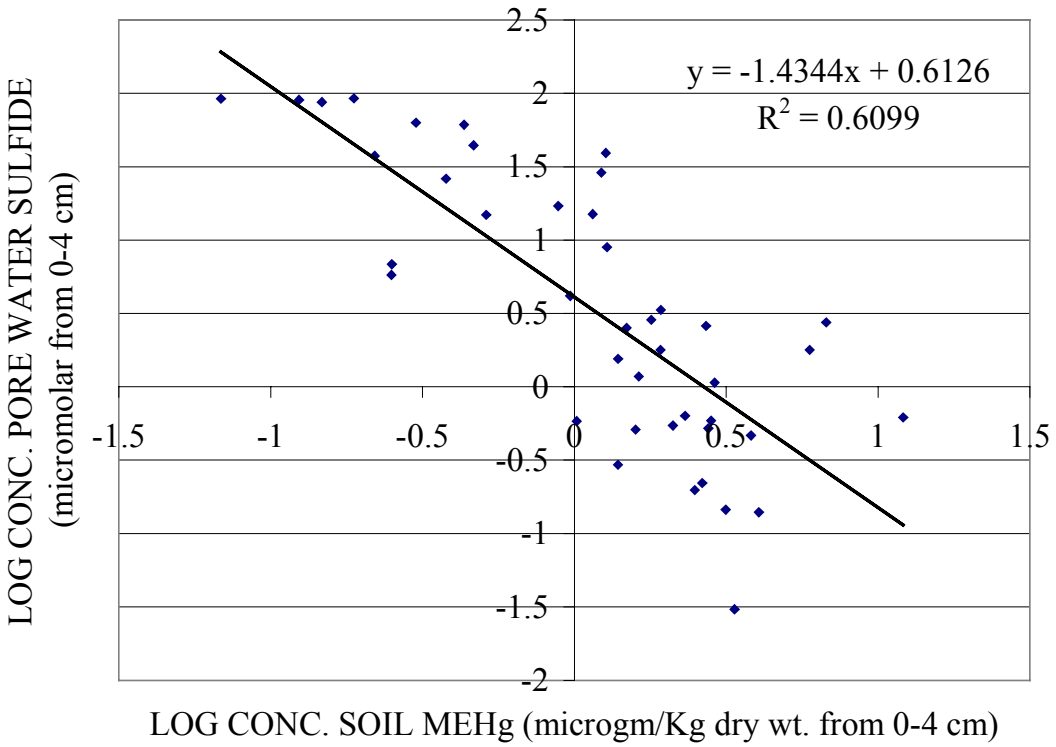

Figure 1. Plot of log concentration of sulfide in soil pore water and MeHg (MeHg) in soil solids

Phosphorus: Phosphorus addition to Everglades soil microcosms or in situ mesocosms has not been shown to stimulate net MeHg production under the same conditions in which sulfate

addition has been shown to stimulate net MeHg production. However, through its direct, positive effect on both live biomass production and dead biomass decomposition, phosphorus can increase the rate of Hg(II) settling to the sediments, while diluting the Hg(II) that reaches the sediments in more rapidly accumulating undecomposed (refractory) biomass (Vaithayanathan et al., 1996). This has the effect of reducing the average concentration of Hg(II) in the water column, where MeHg production generally does not take place, and, all other factors being equal, reducing the rate of MeHg production in the surficial soil. However, the net MeHg production rate measured in Everglades soil cores varies by three orders of magnitude over an Hg(II) concentration range that varies by no more than a factor of five, so something other than the concentration of Hg(II) in surficial soils must be the predominant influence on MeHg production. This other factor is believed to be the concentration of sulfur species that govern the metabolic activity of sulfate-reducing bacteria and the rates of formation and the stabilities of various uncharged and charged Hg(II)-sulfur complexes.

DOC can weaken the influence of an increased peat accretion rate on the settling rate of Hg(II) from the water column by competing with live, dead, and decomposing settling plant particles for Hg(II) and can weaken the influence of sulfur cycle species on the rates of formation and stabilities of various mercury-sulfur complexes (Ravichadran et al, 1998; Ravichadran, 1999). These counter-influences should be manifest in the high-DOC waters of the Everglades. Ironically, the internal production rate of DOC and the attendant concentrations of DOC in surface and pore waters are highest in high-phosphorus waters. In the Everglades, the areas of high water and soil phosphorus levels are also those receiving the highest external load of DOC from EAA runoff, and this external DOC has been shown to have a higher affinity for Hg(II) and MeHg than internally produced DOC, so the counter-influences of DOC should be greatest where the effects of increased rates of settling and sediment dilution are expected to be greatest. This underscores the complexity of mercury biogeochemistry in aquatic ecosystems and the inability of a one-variable model to predict the net MeHg production rate in aquatic ecosystems in general, the Everglades in particular, and the most eutrophic areas of the Everglades most specifically.

MEHG BIOACCUMULATION

The bioavailable MeHg can then enter the food chain by one of three routes. The first is direct transfer to the worms and insects (macroinvertebrates) living on or in the soil/sediment (benthos). The second is direct transfer to the plant-eaters (herbivores) and meat-eaters (carnivores) that ingest soil/sediment in the process of foraging for food in the surficial sediment (bottom-feeders). The third is an indirect route involving transfer of MeHg to the water column, sorption to microscopic plants and animals living in the water column, and thence to the herbivores and carnivores that feed on them. In the Everglades, the shallow water and the highly organic soil/sediment tend to favor direct transfer to benthic macroinvertebrates and thence to their predators and so on up the food chain (Cleckner et al., 1998; Lange et al., 1998, 1999; Hurley et al., 1999; Loftus et al., 1998). Because MeHg is rapidly taken up but only slowly lost (depurated) from aquatic animals, and this loss rate decreases with increasing size (Norstrom et al., 1976; Rodgers, 1994), large sport fish at the top of the food chain can bioaccumulate MeHg as much as 10,000,000 times the concentration in the surrounding water (USEPA, 1997; Lange et al., 1998, 1999).

Factors Influencing MeHg Bioaccumulation

TEMP: Aquatic organism metabolism generally increases with temperature up to the point of onset of thermal stress. With this increase in metabolism is an increase in oxygen demand

(Norstrom et al., 1976). At the same time, DO solubility decreases with increasing temperature (Stumm and Morgan, 1986). This has the effect of increasing the rate at which water is passed across the gill membranes to meet the increased oxygen demand with water depleted in DO. This effect can be exacerbated where aerobic bacteria activity is also high but the re-aeration rate cannot keep up with the increased oxygen demand. This condition often obtains in highly eutrophic waters.

DO: Through its effect on redox potential, iron and manganese speciation, and the sorption of MeHg, DO may influence MeHg bioaccumulation by affecting its bioavailability to small aquatic organisms that absorb MeHg directly across body surfaces. Because a low redox potential likely weakens the affinity of Hg(II) and MeHg for these reduced iron and manganese species surfaces, low DO would be associated with high MeHg bioaccumulation. There is some evidence that iron colloids in the Everglades are competing with Hg(II) and MeHg among the dissolved, DOC-complexed, and particle-sorbed phases, this facilitating transport (Babairz et al., 2001). However, these data are quite limited and further research into this phenomenon is warranted. Conversely, on bioenergetics grounds DO is expected to be inversely correlated with bioaccumulation (R. Harris, TetraTech, personal communication, 2002). This is because low DO requires that all aquatic organisms that breathe via gills must pass more water across their gills to meet their metabolic oxygen demand, and this, in turn, is expected to increase the MeHg uptake at the same time (Norstorm et al., 1976). For large fish, gill uptake of MeHg is trivial, but for small organisms this pathway is important (Rodgers, 1994). Because small organisms are eaten by large organisms, this inverse relationship with DO effect is expected to propagate up the food chain. Because TP is generally inversely correlated with DO in the District's canals and interior marshes, this means that high TP could be associated with high MeHg bioaccumulation via the expected inverse relationship between DO and MeHg bioaccumulation.

DOC: DOC has a strong affinity for Hg(II) (Haitzer et al., 2002) and MeHg (Amirbahman et al., 2002), so DOC competes with particle surfaces for both mercury species, resulting in a reduction in the fractions of the masses of Hg(II) and MeHg that are partitioned to organic particles. In a study of MeHg bioaccumulation by freshwater algae using well-characterized laboratory populations under controlled growth conditions, Miles et al. (2001) observed an inverse relationship between the concentration of DOC and Freundlich isotherm coefficients for MeHg sorbed to algae in the linear concentration region. For aquatic organisms that take up MeHg primarily by ingesting one-celled plants and animals or dead organic particles, a high concentration of DOC will have the effect of reducing MeHg bioaccumulation in those organisms, the organisms that feed on them, their predators, and so on up the food chain. The DOC effect would also be expected to weaken the influence of biodilution on MeHg bioaccumulation via increased production of organic particle, because more of the MeHg will be complexed with DOC and less will be sorbed on organic particles, all other things being equal. Contrary to expectation, in most drainage lakes. A positive correlation has been observed between DOC and MeHg bioaccumulation in fish in a number and variety of temperate lakes (Driscoll et al. 1995). Conversely, DOC has also been demonstrated to reduce significantly the biouptake of MeHg by fish from surface water in a controlled laboratory study (Choi et al., 1998). These apparently contradictory influences can be reconciled by separating lakes into seepage and drainage lakes. Drainage lakes receive the majority of their water from watershed runoff, which carries Hg(I) and MeHg sorbed to suspended solids and complexed with DOC. In seepage lakes, the water comes almost exclusively from groundwater discharge, and in such systems high concentrations of DOC are associated with low concentrations of bioavailable Hg(II) for MeHg production and low concentrations of bioavailable MeHg for bioconcentration, bioaccumulation, and biomagnification.

SO4: No direct effect of sulfate on MeHg bioaccumulation is expected. However, at low sulfate concentrations, sulfate addition stimulates MeHg production, which would lead to higher concentrations of MeHg in the aquatic food chain, all other things being equal. A positive correlation between THg in fish and sulfate in water has been reported (Garcia and Carignan, 1999). At high sulfate concentrations, an inverse correlation with sulfate could emerge, however. A strong inverse relationship has been observed between pore water sulfide and the concentration of MeHg in soil (Gilmour et al., 1998b, 1999) (See **Figure 1)**. For benthic organisms that bioaccumulate MeHg by direct contact with or ingestion of soil MeHg, an inverse correlation between pore water sulfide and the magnitude of MeHg bioaccumulation in fish living in the overlying waters should be expected. However, an even stronger inverse correlation has been observed between pore water sulfide and THg as MeHg in mosquitofish collected from the same Everglades sites (Fink, 2002). High pore water sulfide is likely to be correlated with high sulfate and low DO, all other things being equal (Gilmour, **Appendix 2B-2**).

Cl: The generally high concentrations of chloride (Cl) in the Everglades favor the formation of an uncharged MeHgCl complex at circumneutral pH. Chloride may mediate diffusive uptake of MeHg directly across the surface membranes of small aquatic organisms or across the gill membranes of larger aquatic organisms via the formation of MeHgCl complex. The facilitation of algae uptake of MeHgCl has been observed in marine ecosystems (Mason and Lawrence, 1996). However, in a more recent study of MeHg bioaccumulation kinetics by freshwater algae using well-characterized laboratory populations of algae, Moye et al. (2002) concluded that the preponderance of the evidence supported an active rather than a passive uptake mechanisms for MeHg by freshwater algae, although the predominance of a passive diffusion mechanism could not be ruled out under some circumstances. This is likely to diminish the importance of the formation of a stable MeHgCl complex in mediating MeHg bioaccumulation at the base of the autotrophic food chain, except, perhaps, when algae metabolic activity is very low. However, MeHg bioconcentration in mosquitofish (*Gambusia affinis*) increased with increasing chloride concentration, albeit at concentrations much higher than are generally encountered under ambient conditions (Shin and Krenkel, 1976).

ALK: Alkalinity is the sum of the concentrations of all dissolved carbonate species in water. A number of elements common to natural waters can form carbonate precipitates under ambient conditions. This is especially true of Ca and Mg. In waters high in concentrations of Ca or Mg and low in acidity (neutral to high pH), alkalinity can mediate the removal of trace elements from the water column via co-precipitation with Ca and Mg carbonates or by sorption to the precipitate particle surfaces. In the Everglades, where limerock underlies the peat soil layers and exchange between surficial aquifer water and surface water is common, the waters are of circumneutral pH and near saturation with respect to the precipitation of calcium carbonate or mixed oxyhydroxide-carbonate precipitates. Some organisms, especially the blue-green alga *Schizothrix calcicola*, actively precipitates calcium carbonate in a mucopolysacchride coating to protect it from the damage by the sun's rays and to prevent dessication during the extended dry periods in the Everglades when surface waters disappear altogether in many locations for periods of 90-120 days (J. Grimshaw, personal communication). An inverse relationship between THg in fish and ALK has been reported in a number of studies of northern temperate lakes (Winfrey and Rudd, 1990; Garcia and Carignan, 1999) and Florida lakes (Lange et al., 1993).

pH: Hg(II) bioconcentration in baitfish has been demonstrated to increase with decreasing pH (Tsai et al., 1975). There are a number of studies that detected an inverse relationship between pH and the THg concentration in ambient fish collected form a variety of lakes (Hakanson, 1980; Wiener, 1986; Winfrey and Rudd, 1990; Wiener et al., 1990; Garcia and Carignan). However, Meili (1994) has argued that the apparent inverse correlation between pH and MeHg

bioaccumulation observed in numerous temperate lakes may be spurious due to the influence of primary production on pH. In lakes where P is limiting, one would then expect an inverse relationship between THg in fish and TP in surface water. Nevertheless, pH does mediate a number of physical, chemical, and microbiological processes that govern the transport, fate, and bioaccumulation of MeHg, so attributing all of the effect of pH to its co-correlation with TP is probably over-stated. Some have argued the same point in the opposite direction: that the apparent inverse relationship with primary production in northern temperate lakes is really the effect of pH of mercury transport, fate, and uptake on bioaccumulation, not biodilution.

Hardness: Hardness is expressed as the carbonate equivalents of calcium and magnesium in water. The role of calcium and magnesium in influencing Hg(II) speciation, sorption, and bioaccumulation are set forth in the context of the discussion of ALK and pH influences above. A negative correlation between hardness and MeHg bioaccumulation in top-predator has been observed in Florida lakes (Lange et al., 1993).

TP: Where phosphorus is the limiting nutrient, the rate of primary production of organic biomass is controlled by the phosphorus concentrations in water and sediment. One-celled plants, both individually or in community aggregates (e.g., periphyton mats) and floating plants get their phosphorus almost exclusively from the water, while rooted submergent and emergent plants get most of their phosphorus from the sediment. In lakes, most of the plant biomass is in the form of one-celled plants (i.e., algae, diatoms), with only the littoral zones around the lake perimeter exhibiting significant densities of floating and rooted plants. In wetlands, the opposite is generally true, with most of the area covered by submergent and emergent rooted plants and floating plants, some of the area covered by periphyton mats, and very little of the plant biomass being in the form of free-floating one-celled plants. That being the case, extrapolating the results of lake studies on Hg(II) or MeHg transport, fate, or bioaccumulation to wetlands environments should be carried out only with attention to how these differences will manifest themselves vis-a-vis the applicability of the study results.

With the preceding caveat in mind, an inverse relationship between THg as MeHg in fish and the degree of eutrophication in primarily northern temperate lakes was observed across the United States (D'Itri et al., 1971) and northern Europe (Hakanson, 1980). To explain this phenomenon, Hakanson (1980) speculated that the MeHg concentration in fish was primarily controlled by three factors: pH, Hg(II) flux, and the concentration of suspended solids and developed an empirical model that captured those influences quantitatively. Because the Hg(II) flux is not readily measured in practice, he used the concentration of Hg(II) in the sediments as a surrogate for this value. All three factors are influenced by the rate of primary production, and Hakanson coined the term "biodilution" to explain the apparent inverse relationship between lake productivity and MeHg levels in fish, arguing that where the concentration of biotic particles is high, MeHg concentrations in water, sediment, and fish were low, because of the enhanced rate of removal and dilution through settling and sedimentation, and vice versa.

"Classic" biodilution in lakes is expected to have three primary manifestations:

(1) a sustained increase in primary production per unit area, which dilutes a constant flux of sorbed Hg(II) and MeHg, resulting in a decrease in their concentrations in biomass standing crop, litter, detritus;

(2) a sustained increase in the net settling rate of biomass, which increases the rate of removal of sorbed Hg(II) and MeHg from the water column but dilutes the increased deposition flux of these sorbed mercury species in a sustained increased flux of accreting sediment;

(3) a sustained increase in the densities and growth rates of herbivores and carnivores supported by sustained increase in primary production, resulting in the growth dilution of the bioaccumulating MeHg (Norstrom et al., 1976).

Miles et al. (2001) observed an inverse relationship between the concentration of phosphorus and Freundlich isotherm coefficients for MeHg sorbed to algae undergoing exponential growth rates in the linear concentration region of MeHg sorption. However, the effect of phosphorus addition was more complicated for one species of algae that changed its cell structure as well as growth rate in response to higher phosphorus concentrations. The biodilution phenomenon is not limited to mercury in specific or metals in general but has also been observed for organic compounds (Dachs et al., 2000).

As with lakes, all other things being equal, the addition of phosphorus to a phosphorus-limited wetland ecosystem will increase the rate of primary production up to a point. The upper limit to this effect is reached when the velocity of the enzyme-mediated, rate-limiting step reaches saturation (Monod, 1942) or another environmental factor becomes limiting (Carlson, 1980; Brezonick et al., 1984) or toxic. Such other limiting factors include self-shading. Toxic factors include plant exudates intended to exclude competitors and pore water sulfide. This increased primary production will then translate into a higher organic particle settling rates and sediment accretion rates (Chimney and Moustafa, 1999), higher Hg(II) and MeHg settling rates (Ambrose and Araujo, 1998), and a lower Hg(II) concentration in the more rapidly accreting peat soil (SFWMD, 1995-1999), as was observed along the WCA-2A nutrient gradient (Vaithiyanathan et al., 1996). However, DOC competes with organic particles for Hg(II) and MeHg, so at high DOC concentrations, more of the Hg(II) and MeHg in the system is complexed with DOC than sorbed to particles, which must necessarily reduce the organic particle-mediated flux of Hg(II) and MeHg to the sediment. This is likely to translate into a lower concentration of Hg(II) in the sediment. All other things being equal, this would decrease MeHg production, but, as has been noted above, the net rate of MeHg production is more strongly influenced by pore water DOC, sulfate, and sulfide concentrations. In addition, it should be noted that where MeHg is being produced almost exclusively in the surficial sediment, the concentration of MeHg there is determined primarily by the rate of its production from Hg(II), not its deposition flux from the overlying water column. Conversely, higher DOC concentrations should have the effect of increasing the concentrations of Hg(II) and MeHg in the water column and in the filtered fraction of surface water. In systems where net MeHg production occurs primarily in the water column, high DOC concentrations may enhance the rate of MeHg production by holding Hg(II) up in the water column until it can be absorbed and methylated by the appropriate bacteria.

In addition to its influence on DOC and organic particle production, TP also has indirect influences of MeHg bioaccumulation via the carbon and oxygen cycles. The sustained increase in the loading rate of P to a P-limited system will increase plant densities, production rates, and aerobic and anaerobic decomposition rates up to a point. This will cause the average DO concentration in the water to decrease, with a concomitant shift to micro- and macroorganisms with a greater tolerance for a low-DO environment. Sediment DOC, carbon dioxide, methane, and hydrogen sulfide production rates will also tend to increase (Drake, 1994). DOC and pore water sulfide are expected to have an inverse relationship with MeHg bioaccumulation, while low DO should increase MeHg bioaccumulation by increasing the rate at which water must be passed across the gills to meet the organism's oxygen demand in proportion to caloric intake and thus the rate of uptake of MeHg across the gills. These effects are discussed in greater detail in the preceding sections on the influence of DO, DOC, and sulfate on MeHg bioaccumulation.

EMPIRICAL MODELS OF MERCURY BIOACCUMULATION

The problem of MeHg bioaccumulation in aquatic ecosystems has been the subject of numerous studies over the last 30 years in northern Europe, Canada, and the United States. Originally thought to be a byproduct of watershed and lake acidification in the poorly buffered lakes of the northern tier of states, it is now recognized that mercury bioaccumulation in aquatic ecosystems is a nationwide problem, and the worst cases are now located in the southeastern U.S. To better understand what, in fact, was causes a lake to be susceptible to a mercury problem, a number of short-term and long-term studies have been undertaken by various federal and state agencies. As a consequence, there are a large number of data sets amenable to empirical analysis.

Johnels et al. (1967) first noted that nutrient-enriched or eutrophic lakes were less likely to exhibit a MeHg bioaccumulation problem than unenriched or oligotrophic lakes. This observation was subsequently confirmed by D'Itri et al. (1971) and Hakanson (1974). This was attributed to the buffering or dilution effect caused by an increase in suspended solids of biological origin.

Subsequently, Hakanson (1980) concluded from the body of evidence then available that the average MeHg concentration in fish from northern temperate and sub-Arctic lakes was related qualitatively to the pH, trophic state, and degree of mercury contamination of the system. To quantify these interrelated functional relationships, he arrived at the following simple mathematical formula:

$$F(Hg) = [4.8 \log (1 + Hg50/200)]/[(pH-2) \times \log BPI]$$

Hg50 = weighted mean Hg-content of surface sediments, 0-1 cm, in ng/g dry solids

pH = negative logarithm of the molar concentration of hydrogen ion

BPI = the bioproduction index

The fish to which this formula is applicable is a 1-Kg northern pike or equivalent. The sediment THg concentration was adopted as a surrogate for the input flux of Hg(II), because the concentration in sediment is determined by the deposition flux and the sediment accretion rate (Vaithiyanathan et al., 1996).

In the late 1980s and early 1990s, Lange et al. (1993) carried out a study of the relationship between water quality and THg in largemouth bass standardized to age class 3 years in 53 Florida lakes. The authors found a strong ($r > 0.64$) inverse correlation between THg in largemouth bass and surface water pH, a moderate ($0.36 < r < 0.64$) inverse correlation with alkalinity, chlorophyll a, hardness, and TKN, in that order, a weak ($r < 0.36$) negative correlation with total phosphorus, and a weak positive correlation with lake surface area and secchi depth. No predictive empirical relationships were generated by the authors, however.

There are no wetlands in the database from which Hakanson (1980) developed his relationship. Nevertheless, PTI (1994) applied the biodilution hypothesis and Hakanson's formula to the problem of evaluating the MeHg risks to fish-eating wildlife following the restoration of the already impacted areas of the northern Everglades and concluded that a reduction in water column total phosphorus would cause an ecologically significant increase in MeHg

bioaccumulation in such areas. A limited set of site-specific data obtained by KBN (1994) was also introduced to support this concern.

To evaluate the effect of acid rain on MeHg watershed transport and bioaccumulation, Richardson et al. (1995) derived or reproduced simple regression relationships between routinely measured water quality variables and the bioaccumulation of MeHg in large, top-predator fish using published data for a number of northern temperate lakes studies. The following equations were evaluated:

log10 (trout THg) = -1.072 + 0.132*(DOC)

$p \leq 0.0001$, $r^2 = 0.37$, n = 61 lakes Sorenson et al. (1990)

log10 (pike THg) = 3.5(+/-0.6) + 0.65(+/-0.18)*log 10(TOC) –0.21 (+/-0.07)*pH

$p < 0.05$, $r^2 = 0.37$, n = 53 lakes McMurtry et al. (1989)

(walleye THg) = 3.71 – 0.46*pH

$p < 0.05$, $r^2 = 0.49$, n = 48 specimens from 13 lakes Wiener et al. (1990)

PTI, Inc. (1994, 1995a) obtained a limited set of mosquitofish THg concentration data collected in September 1993 and March 1994 by USEPA Region 4 along the "F" research transect in the WCA-2A downstream of the S-10 structures (**Figure 2**), paired it with the corresponding total phosphorus water column concentration data collected by USEPA at the same time, and obtained a nonlinear equation as the best fit to the data. The authors did not perform an exploratory data analysis to identify the strongest predictors of THg in mosquitofish; nor did the authors give consideration to any of the other empirical relationships published in the peer-reviewed scientific literature summarized above. Instead, the authors forced the relationship with total phosphorus in the water column, based on the assumption that MeHg bioaccumulation along the nutrient gradient was being dictated by biodilution processes and that phosphorus was the limiting nutrient. The analysis was subsequently repeated with the same mosquitofish data but replacing the one-time USEPA water column total phosphorus concentration results with the average water column TP concentrations collected by the District for the same period (PTI, 1995b).

However, no consideration was given to the THg concentration in sediment or pH in water that were identified by Hakanson (1980) as important determinants of MeHg bioaccumulation in lakes and present as variables in his predictive formula. Nor was consideration given to the possibility that some other factor, i.e., photosynthetically active radiation (PAR), was limiting along the WCA-2A nutrient gradient due to the invasion of cattail,

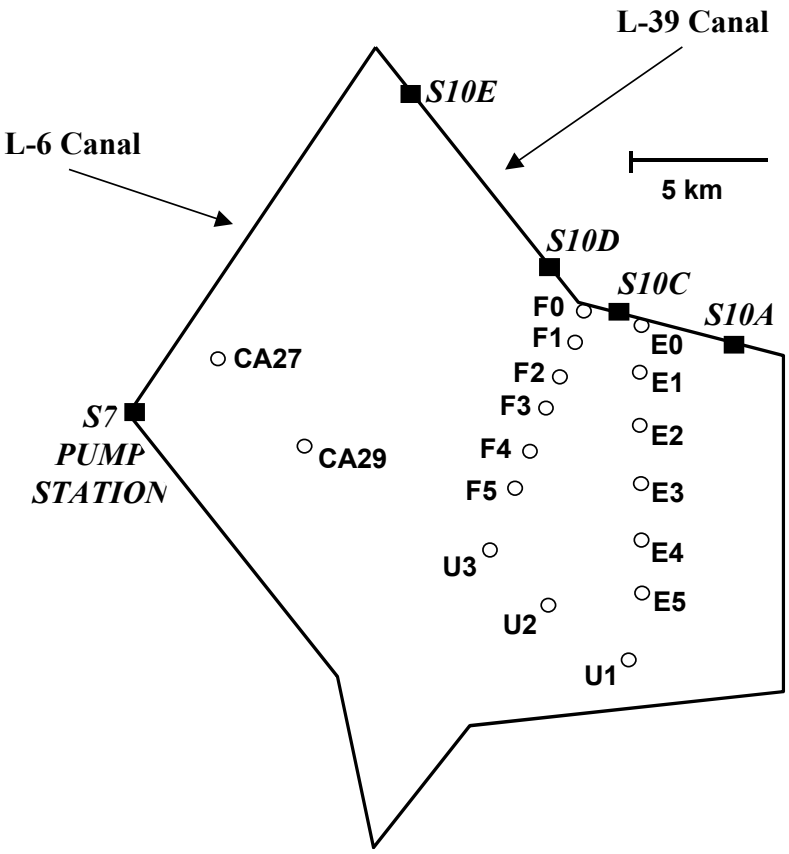

Figure 2. Nutrient impact research sites along the "E" and "F" Transects in Water Conservation Area 2A in the northern Everglades.

forming a canopy of live and dead plants, the density of which decreases along the decreasing nutrient gradient (Grimshaw et al., 1997; McCormick et al., 1999; Fink and Rawlik, 2000). Contrary results produced in valid studies were also not discussed. For example, where eutrophication was artificially induced with phosphorus addition in a mesocosm study carried out in an impoundment of the English-Wabigoon River, the biodilution effect was more than offset by increased MeHg production at moderate phosphorus concentrations (Rudd and Turner, 1983).

In addition to the hypothesized and observed inverse relationship between water column phosphorus and mosquitofish THg along the WCA-2A nutrient gradient, an inverse relationship between pore water sulfide and mosquitofish THg has been observed in a five-year study of 13 interior Everglades marsh sites (Gilmour et al., 1998). It has been hypothesized that where sulfate input is high, and dissolved oxygen (DO) is low, pore water sulfide can build up to concentrations that inhibit MeHg production by a mechanism that has yet to be fully elucidated (Gilmour et al., 1998; Benoit et al., 1999; 2000; Jay et al., 2000).

Beyond its hypothesized direct effect on MeHg production, together with low DO, high sulfide in pore water and overlying surface water could foster the replacement of pollution-intolerant, primarily autotrophic and pelagic aquatic species, with pollution-tolerant, primarily saprotrophic and benthic aquatic species. These changes in community composition are likely to translate into corresponding changes in food web structure and the relative contributions of the benthic and pelagic food webs to bioaccumulation at higher trophic levels without any manifestation of a "classic" biodilution effect.

Subsequently, PTI, Inc, now Exponent, Inc., obtained a revised nonlinear equation using a new approach for averaging the total phosphorus concentrations in the 1994 data sets and for analyzing the data (Exponent, 1998). That equation is:

Regression: Mosquitofish THg (ug/Kg) = 5,316 x TP(ug/L) ^-1.262

Upper 95th percentile C.I.: Mosquitofish THg (ug/Kg) = EXP(10.467-2.29*[ln TP] + 0.155 [ln TP]^2)

EXPLORATORY DATA ANALYSIS USING DISTRICT MONITORING DATA

In this section an exploratory data analysis is carried out on District data, pairing fish mercury concentrations with the corresponding surface water, pore water, or soil constituent concentrations collected in the same vicinity as the fish. After summarizing the general sampling and analysis procedures for THg (THg) as MeHg (MeHg) in fish and unfiltered and filtered ultra-trace THg and MeHg in surface water, the sampling site is described, data censorship criteria and analysis methods are set forth, and the results of the exploratory data analysis are tabulated and discussed. Three sites are analyzed in detail: the L-7 Canal Site (ENR 004), the "F" Transect Sites along a well-studied nutrient gradient in Water Conservation Area 2A (WCA-2A), and four permit compliance monitoring sites in the interior marshes of WCA-1, WCA-2A , WCA-3A, and Everglades National Park (ENP).

GENERAL SAMPLING PROCEDURES

Unfiltered and filtered surface water samples were collected for THg (THg) and MeHg (MeHg) using the "clean hands-dirty hands" technique. During sample collection, both "clean hands" and "dirty hands" wore unpowdered, wrist-length plastic gloves, but 'clean hands" only touched equipment and surfaces that had been prepared in a low-mercury laboratory environment (e.g., sample bottles, filters), while "dirty hands" opened and closed the coolers, removed and returned the double bagged sample bottles from and to the coolers, and opened the outer bag so that "clean hands" could open the inner bag and remove the sample bottle for sample collection. Unfiltered water was collected by drawing a subsurface (10-15 cm) sample through an acid pre-cleaned 100-micron Nitex ® pre-screen, acid pre-cleaned Teflon tubing, and a short section of acid pre-cleaned Masterflex tubing using a peristaltic pump. The sampling train was equilibrated with *in situ* water by pumping for a minimum of two minutes. Then the acid pre-cleaned Teflon ® sample bottle and cap were rinsed three times with *in situ* water drawn through the sampling train prior to sample collection. The sample bottle was returned to the inner bag and sealed by "clean hands" and the outer bag was sealed and returned to the cooler by "dirty hands." Unpreserved water samples were stored on blue ice, shipped by overnight carrier to the analytical laboratory, and preserved for analysis within 48 hours. The analysis of the preserved samples was then carried out within 28 days.

In October 1997, the District switched ultra-trace mercury analysis laboratories from the Florida Department of Environmental Protection to Frontier Geosciences. From August 1994 until July 1998, filtered samples were collected by attaching a 0.4 micron filter manufactured by Gelman Scientific without acid pre-cleaning. In April 1998, it was determined that the Gelman filters were occasionally contaminated with Hg(II) at significant levels, resulting in the filtered THg exceeding that of the unfiltered THg by a statistically significant amount (Rumbold, 1998). In July 1998, a pre-cleaned 0.4-micron Meissner ® quartz fiber filter was substituted for the Gelman filter. The THg results for filtered samples collected prior to July 1998 were flagged as potentially contaminated. There was no evidence that the filtered MeHg samples were compromised prior to July 1998, and so these data were not flagged.

Mosquitofish were collected with a dip net from levee banks or docks, and, initially, seven mosquitofish were randomly selected for analysis from the several dozens collected. By April 1995, it had been determined that there was a trimodal THg concentration distribution in mosquitofish collected in the ENR Project inflow, outflow, interior culverts and marshes, and the

L-7 Canal: small fish, which represent primarily juveniles, and large fish, which represents primarily pregnant females, exhibited significantly different averages and standard deviations from those of mid-size fish. The breakpoint between the small and medium fish was determined to be 0.07g, while that between large and medium fish was determined to be 0.29g (P. Rawlik, SFWMD, personal communication). It was also determined that the average of ten, mid-size mosquitofish subsampled at random from a sample population and analyzed individually produced the same average concentration as the average of five subsamples of a homogenate of a multi-fish composite of the remaining fish in that sample population. Thereafter, between 75-250 mosquitofish were collected at each site, the fish were sorted into small, medium, and large fish, the small and large fish were frozen, and the medium fish were homogenized and the homogenate was subsampled five times for individual analysis for THg. The unused portion of the homogenate was archived for reanalysis or split analysis in an inter-laboratory round-robin.

Twenty sunfish (*Lepomis sp.*) and 20 largemouth bass (*Micropterus salmoides*) were collected at each of ten interior marsh sites by electroshocking, measured, weighed, and stored on ice until returned to the laboratory. Samples were subsequently frozen until processed. Individual whole sunfish were homogenized, refrozen, and shipped frozen to the analytical laboratory for acid digestion and THg analysis by the FDEP laboratory using standard methods. The heads of the largemouth bass were removed at the time of processing, the otoliths were removed for aging, and the otoliths were subsequently cleaned, dried, polished, and viewed under the microscope to count age rings. After thawing, the bass is filleted and a section of the bass muscle is cut away by dicing. The diced muscle is then shipped frozen to the FDEP analytical laboratory for acid digestion and THg analysis using standard procedures.

STUDY SITE: L-7 CANAL (ENR 004)

The L-7 Canal is one of a system of over 1200 miles of canals and associated pumps and weirs built by the U.S. Army Corps of Engineers that regulate the direction and magnitude of stormwater and seepage flow in south Florida. The modern flood control and water supply system was begun in 1948 and was essentially complete by the mid-1960s (SFWMD, 1992). The Southern Florida Flood Control District (now the South Florida Water Management District, hereinafter referred to as the District) was created by an act of the Florida Legislature to be the local sponsor for the Corps project. Stormwater that collects in the 800,000-acre Everglades Agricultural Area (EAA) via a series of secondary canals is recirculated for reuse during dry periods. Stormwater in excess of what can be used/reused is eventually pumped into one of the District's four primary canals that pass through the EAA and thence into the northern Everglades. Shortfalls in the EAA water supply are made up by releases from Lake Okeechobee via three primary weirs.

Prior to 1972, most of the EAA stormwater was pumped into Lake Okeechobee for wet season storage for dry season reuse, less what was lost to evapotranspiration. Some "backpumping" of EAA stormwater still occurs under grandfathered permits. The stormwater runoff contains nutrients, dissolved organic carbon, and trace metals leached from the primarily peat soils of the EAA. Prior to 1992, the quality of this water was unregulated. Since then, the District has passed Best Management Practice rules that require a 25% reduction of the load of total phosphorus (TP), because P has been determined to be the limiting nutrient in the Everglades (SFWD, 1992). In addition, the District is constructing nearly 50,000 acres of constructed wetlands referred to as Stormwater Treatment Areas (STAs) to treat all but the most extreme volumes of EAA runoff prior to discharge into the northern Everglades. The target

outflow concentration for the STAs is < 50 ppb on a flow-weighted annual average. That target is being met by all but one of the STAs constructed to date. By 2006 the District is to achieve compliance with the proposed numerical Class III Water Quality Standard for total phosphorus of 10 ppb.

The L-7 Canal transports EAA stormwater runoff pumped from the S-5A Pump Station to the L-39 canal, where it commingles with EAA runoff pumped from the S-6 Pump Station and then flows into the eastern portion of WCA-2A via the S-10 culverts. After the Everglades Nutrient Removal (ENR) Project began operation in August 1994, about one-third of the water that would have been discharged untreated directly through the S-5A Pump Station into the L-7 Canal and thence into the Arthur R. Marshall Loxahatchee National Wildlife Refuge (WCA-1) via canal overflow was treated prior to discharge into the L-7 Canal about 10 km downstream of the S-5A Pump Station. A reference station was established about 3 km upstream of the Outflow Pump Station to evaluate the net effect of the ENR Project discharge on receiving water quality. The site was initially accessed by airboat, but a floating walkway was eventually built that allowed access across the L-7 Levee to the L-7 Canal on foot. At ENR 004, the L-7 Canal is roughly 100 ft across and 15 ft deep. It is sprayed routinely with herbicides to control bank weeds and floating aquatic vegetation. It is dredged infrequently, because the rate of organic sediment accumulation is low.

Traditional water quality parameters were monitored from April of 1993 until the ENR Project was subsumed by STA-1W in April 1999. Monitoring of unfiltered THg and MeHg began in August 1994 and filtered THg and MeHg were added in January 1995, while monitoring of mosquitofish began in December 1994. Although the ultra-clean mercury sampling for ultra-trace mercury analysis was not carried out concurrently with the collection of samples to be analyzed for the other water quality constituents, the mercury sampling was scheduled so that the samples were generally collected the same, the immediately preceding, or the immediately following day but no more than 48 hours before or after. The ENR 004 L-7 Canal Site represents the longest continuous sampling record for ultra-trace mercury species, other water quality constituents, and mosquitofish. No sediment or pore water were collected at ENR 004, however. The study site is depicted in **Figure 3.**

Data Censorship and Reduction

All THg and MeHg results below the method detection limit (MDL) were eliminated from further consideration. All data above the method detection limit (MDL) but below the practical quantitation limit (i.e., 3 times the MDL) were retained for this analysis. A datum fatally flagged because it failed a field quality control (QC) criterion was not rejected out of hand, due to the limited number of data pairs available for this analysis. Most of the data were fatally flagged because the relative percent difference (RPD) of the field duplicate of the sample exceeded 40%. The 40% value was adopted because it represented the RPD that was routinely achievable (mean plus two standard deviations) using the equipment and analytical instruments at the time. However, because of bottle contamination problems with Teflon bottles revealed subsequent to the termination of the ENR Project monitoring program, there is no way to determine *a priori* whether the problem lay with irreproducible sampling technique or because of bottle contamination. The switch to glass bottles in 2001 has demonstrated that replicate field sampling technique following the “clean hands-dirty hands” protocol is highly reproducible. Thus the decision was made not to reject data based solely on imprecision fatal flags. However, because filtering can introduce contamination, if the filtered THg or MeHg was greater than unfiltered

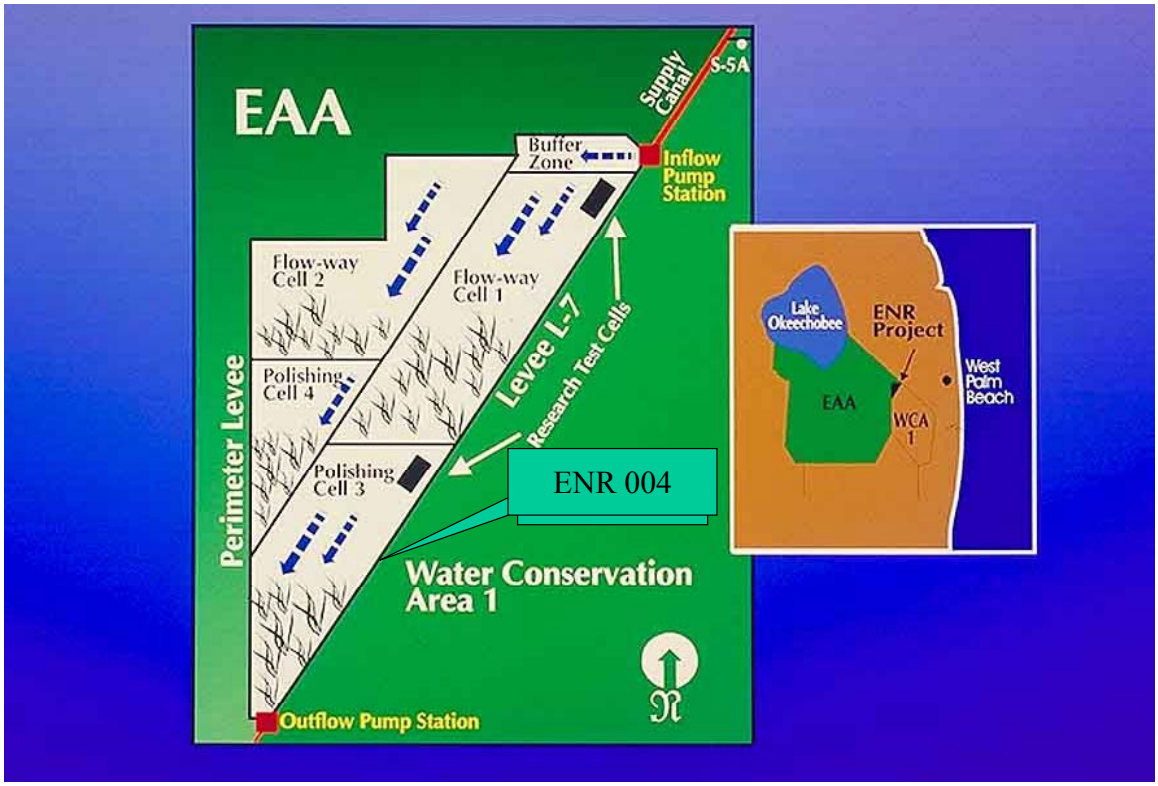

Figure 3. ENR Project long-term study site in the L-7 Canal at ENR 004 upstream of the Outflow Pump Station

THg or MeHg (i.e., the ratio of filtered to unfiltered > 1), the datum was rejected so that there would be no negative concentration values. A value of 1 was not rejected *a priori*, however, because it is possible that such a large fraction of THg or MeHg was in the dissolved state that the difference between filtered and unfiltered was negligible. (In fact, statistically, one should expect some ratios greater than 1 when the concentration is below the PQL but above the MDL, but it was decided that this might introduce additional uncertainty into the analysis, so the rejection criterion was made more stringent.)

Unfiltered Hg(II), Hg(II), was calculated by difference as unfiltered THg, THg-U less unfiltered MeHg, MeHg-U. Likewise, filtered Hg(II), Hg(II)-F, as THg-F less MeHg-F. Hg(II)-P and MeHg-P were calculated as Hg(II)-U less Hg(II)-F and MeHg-U less MeHg-F, respectively. Despite the absolute rejection of any filtered sample greater than the unfiltered sample for both THg and MeHg, on occasion the calculated values for Hg(II)-F exceeded Hg(II)-U. In this case, the Hg(II)-F and Hg(II)-U values were not deleted, but the negative Hg(II)-P value was deleted. In addition, if the ratio of Hg(II)-F to Hg(II)-U exceeded 25% (i.e., a Hg(II)-F/Hg(II)-U > 1.25), the results were rejected. This in recognition of the fact that the propagated uncertainty in the calculated value is higher than uncertainty in the data used in its calculation. However, in all but one instance the ratio was less than 10%.

After censoring the data in this way, the following number of sample pairs were available for analysis, as displayed in **Table 1**.

Table 1. Number of data pairs for exploratory data analysis for L-7 Canal mercury monitoring program at ENR 004

Parameter		N (number of samples)
Hg(II)-U		59
MeHg-U		60
Hg(II)-F		44
MeHg-F		46
Hg(II)-P		40
MeHg-P		44
Fraction MeHg-U/THg-U		58
Fraction MeHg-F/THg-F		43
Fraction MeHg-P/THg-P		34
Fraction Hg(II)-F/Hg(II)-U		42
Fraction Hg(II)-P/Hg(II)-U		42
Fraction MeHg-F/MeHg-U		44
Fraction MeHg-P/MeHg-U		44
Mosquitofish THg		18
Mosquitofish THg vs MeHg-U		18
Mosquitofish THg vs MeHg-F		15
Mosquitofish THg vs MeHg-P		14

Data Analysis Methods

To identify potentially significant influences on MeHg production and bioaccumulation, an exploratory linear regression analysis was carried out on the quarterly mosquitofish THg concentration data (dependent variable) paired with corresponding water quality data collected at the same time (t) (independent variable). The analysis was then repeated with the following water quality data transformations: average of t, t-1; average of t, t-1,t-2; average of t-1, t-2, and t-3; the average of t-2, t-3, and t-4; and the natural logarithmic transformation of each. The analysis was then repeated with the ratio of the mosquitofish THg concentration to the unfiltered MeHg concentration in water collected at the same time (BCF-U) paired with the water quality data as per the preceding. The analysis was again repeated with the ratio of the THg in mosquitofish to filtered MeHg in water (BCF-F) and the ratio of mosquitofish THg concentration to particulate MeHg (BCF-P), where particulate MeHg is calculated as the difference between MeHg-U and MeHg-F. To evaluate the potential for co-correlations to mislead one's interpretation of the results, the co-correlations between surface water parameters were also calculated for the period of record.

Results and Discussion

MeHg production was not measured directly in the L-7 Canal at ENR 004 or anywhere else, so it had to be inferred from the ratio of MeHg-U to THg-U, based on the assumption that that ratio is higher when internal production is high and lower when internal production is low relative to the inflow ratios. However, this would require monitoring of an upstream site that captures the concentrations of THg and MeHg in EAA runoff prior to entering the L-7 Canal, and such monitoring did not occur. Nevertheless, because EAA runoff is the source of virtually all of the water in the L-7 canal with but a few, short-term exceptions when Lake Okeechobee water predominated, a strong correlation between the ratio of MeHg-U to THg-U could reflect the effect on MeHg production in the EAA secondary canals prior to discharge. Thus, while the use of this ratio to infer the influence of water quality parameters on MeHg production within the L-7 Canal has been compromised, its use in inferring what affects MeHg production in the EAA system probably has not. This robustness of this inference is reduced by transport and fate process that alter that ratio in the District's primary canal system prior to arriving at ENR 004. The recirculation time and travel times in the EAA system and the District's primary canals prior to arriving at ENR 004 are also unquantified, so these cannot be used as potentially influential parameters in the regression analysis.

Table 2 displays the correlation coefficients for the regression analysis of Hg(II)-U, -F, and –P; MeHg-U, -F, and –P; fraction MeHg-U/THg-U, MeHg-F/THg-F, and MeHg-P/THg-P; and Hg(II)-F/Hg(II)-U, Hg(II)-P/Hg(II)-P, MeHg-F/MeHg-U, and MeHg-P/MeHg-U. Table 3 summarizes the results for the natural logarithmic (LN) transformation of the same mercury species concentrations and their ratios. Because the strongest correlations were between mosquitofish THg and the average of the water quality values for the three months preceding the mosquitofish collection (t-1, t-2, and t-3), only these results are displayed in **Table 4**. The results of the co-variance analysis among the various water quality parameters (t,t) are displayed in **Table 5**.

Table 2. Pearson correlation analysis of Hg(II) and MeHg species concentrations and their ratios vs surface water constituents in the L-7 Canal at ENR 004 (untransformed data)

	MeHg -U	Hg(II) -U	MeHg -F	Hg(II) -F	MeHg -P	Hg(II) -P	fraction MeHg -U/T	fraction MeHg - U/T	fraction MeHg -P	fraction Hg(II) -F/U	fraction Hg(II) -P/U	fraction MeHg -F/U	fraction MeHg -P/U
TEMP	0.17	0.15	0.21	0.23	0.22	0.18	0.17	0.22	0.27	0.24	0.23	0.23	0.21
DO	0.05	0.06	0.03	0.02	0.03	0.10	0.05	0.03	0.04	0.04	0.06	0.02	0.04
pH	-0.07	-0.02	-0.09	-0.07	-0.08	-0.06	-0.07	-0.09	-0.11	-0.10	-0.09	-0.09	-0.08
TSS	-0.07	-0.02	-0.09	-0.07	-0.08	-0.06	-0.07	-0.09	-0.11	-0.10	-0.09	-0.09	-0.08
TP	0.17	0.22	0.20	0.22	0.20	0.24	0.16	0.21	0.21	0.19	0.20	0.20	0.20
CA	0.17	0.19	0.22	0.24	0.24	0.24	0.17	0.27	0.31	0.24	0.23	0.25	0.23
MG	0.17	0.19	0.23	0.25	0.24	0.23	0.17	0.30	0.31	0.24	0.24	0.24	0.23
CL	0.23	0.24	0.30	0.28	0.30	0.27	0.23	0.33	0.32	0.29	0.29	0.30	0.29
SO4	0.11	0.12	0.15	0.15	0.15	0.12	0.11	0.18	0.18	0.15	0.14	0.16	0.15
ALK	0.20	0.21	0.25	0.28	0.27	0.25	0.19	0.31	0.35	0.27	0.27	0.28	0.26
TN	0.00	0.04	0.00	0.02	0.00	0.02	-0.01	0.01	0.01	0.00	0.00	0.00	-0.01
DOC	0.10	0.11	0.14	0.16	0.15	0.11	0.09	0.17	0.19	0.15	0.14	0.16	0.13
TDS	0.22	0.22	0.29	0.30	0.29	0.27	0.21	0.34	0.36	0.30	0.29	0.30	0.28

Table 3. Pearson correlation analysis of Hg(II) and MeHg species concentrations and their ratios in vs surface water constituents in the L-7 Canal at ENR 004 (LN transformed data)

	LN MeHg -U	LN Hg(II) -U	LN MeHg -F	LN Hg(II) -F	LN MeHg -P	LN Hg(II) -P	LN fraction MeHg -U/T	LN fraction MeHg - U/T	LN fraction MeHg -P	LN fraction Hg(II) -F/U	LN fraction Hg(II) -P/U	LN fraction MeHg -F/U	LN fraction MeHg -P/U
TEMP	0.36	-0.05	0.51	0.30	0.01	-0.20	0.34	0.42	0.15	0.18	-0.25	0.46	-0.37
DO	-0.63	-0.07	-0.64	-0.33	-0.13	0.20	-0.50	-0.55	-0.22	-0.28	0.21	-0.52	0.46
pH	-0.31	-0.09	-0.19	-0.21	-0.18	0.20	-0.21	-0.10	-0.27	-0.23	0.31	-0.13	0.04
TSS	0.06	0.37	0.01	0.36	0.23	0.27	-0.24	-0.17	-0.14	-0.13	0.16	-0.32	0.24
TP	0.63	0.54	0.52	0.43	0.47	0.19	0.20	0.34	0.13	-0.07	0.01	0.06	-0.04
CA	0.36	0.17	0.40	0.17	0.00	-0.01	0.18	0.40	-0.13	0.03	-0.07	0.44	-0.38
MG	0.25	0.11	0.27	0.10	-0.01	-0.06	0.12	0.29	-0.06	0.03	-0.08	0.30	-0.22
CL	0.07	0.01	0.06	-0.12	-0.06	-0.07	0.03	0.16	-0.01	-0.04	0.00	0.12	-0.06
SO4	0.32	0.13	0.32	0.15	0.11	-0.09	0.17	0.32	0.07	0.08	-0.10	0.29	-0.16
ALK	0.35	0.11	0.40	0.15	0.04	-0.17	0.21	0.43	0.10	0.09	-0.24	0.47	-0.30
TN	0.41	0.32	0.39	0.33	0.23	0.10	0.18	0.30	0.02	0.04	-0.01	0.24	-0.08
DOC	0.55	0.15	0.62	0.27	0.08	-0.15	0.35	0.60	0.10	0.10	-0.21	0.59	-0.50
TDS	0.28	0.09	0.32	0.10	0.07	-0.15	0.16	0.35	0.12	0.06	-0.17	0.33	-0.16

Table 4. Co-variance analysis of surface water constituents in the L-7 Canal at ENR 004

	TEMP	DO	pH	TSS	TP	CA	CL	SO4	ALK	TN	DOC
TEMP	1.00	-0.55	-0.10	0.09	0.19	0.20	0.13	0.14	0.19	0.15	0.32
DO	-0.55	1.00	0.55	0.06	-0.44	-0.43	-0.21	-0.39	-0.39	-0.36	-0.54
pH	-0.10	0.55	1.00	0.02	-0.39	-0.13	0.07	-0.12	-0.08	-0.16	-0.22
TSS	0.09	0.06	0.02	1.00	0.37	0.11	-0.06	0.09	0.07	0.31	0.10
TP	0.19	-0.44	-0.39	0.37	1.00	0.55	0.31	0.54	0.50	0.67	0.56
CA	0.20	-0.43	-0.13	0.11	0.55	1.00	0.72	0.87	0.94	0.71	0.89
MG	0.15	-0.31	-0.04	0.09	0.48	0.94	0.84	0.91	0.94	0.69	0.85
CL	0.13	-0.21	0.07	-0.06	0.31	0.72	1.00	0.74	0.76	0.43	0.64
SO4	0.14	-0.39	-0.12	0.09	0.54	0.87	0.74	1.00	0.89	0.74	0.83
ALK	0.19	-0.39	-0.08	0.07	0.50	0.94	0.76	0.89	1.00	0.68	0.92
TN	0.15	-0.36	-0.16	0.31	0.67	0.71	0.43	0.74	0.68	1.00	0.73
DOC	0.32	-0.54	-0.22	0.10	0.56	0.89	0.64	0.83	0.92	0.73	1.00
TDS	0.18	-0.38	-0.05	0.04	0.49	0.88	0.85	0.92	0.96	0.67	0.88

Table 5. Pearson correlation analysis of mosquitofish THg vs surface water constituents in the L-7 Canal at ENR 004

	THg Fish	LN THg Fish	BCF MeHg-U	LN BCF MeHg-U	BCF MeHg-F	LN BCF MeHg-F	BCF MeHg-P	LN BCF MeHg-P
(-1,-2,-3)								
TEMP	-0.13	0.03	-0.48	-0.37	-0.46	-0.36	-0.20	-0.14
DO	0.29	0.21	0.65	0.62	0.44	-0.17	-0.25	-0.09
PH	0.31		0.43		0.38		0.21	
TSS	-0.05	0.11	-0.19	0.04	-0.24	-0.04	-0.22	0.03
TP	-0.57	-0.56	-0.66	-0.71	-0.74	-0.84	-0.30	-0.47
CA	-0.34	-0.40	-0.39	-0.51	-0.50	-0.62	-0.17	-0.30
CL	-0.26	-0.30	-0.47	-0.47	0.22	0.53	-0.18	-0.39
SO4	-0.46	-0.48	-0.48	-0.52	0.22	0.69	-0.27	-0.45
ALK	-0.40	-0.44	-0.47	-0.56	-0.63	-0.72	-0.29	-0.44
TN	-0.26	-0.36	-0.20	-0.27	-0.09	0.54	-0.25	-0.34
DOC	-0.47	-0.47	-0.62	-0.71	-0.74	-0.81	-0.26	-0.42
TDS	-0.41	-0.45	-0.55	-0.58	0.12	0.72	-0.24	-0.46

Factors Influencing Hg(II) and MeHg Transport

To the extent that EAA runoff makes the dominant contribution to the Hg(II) and MeHg loads in the District's upper canal system, factors that minimize Hg(II) and MeHg sorption to settling particles or stationary phases (e.g., stationary sediment, attached algae mats, bacteria microfilms of exposed stationary surfaces) will increase the concentration at ENR 004. Total dissolved solids (TDS), chloride (Cl), alkalinity (ALK), total phosphorus (TP), and temperature (TEMP) are the "strongest" of the weak correlates with the absolute concentration of Hg(II)-U and MeHg-U. For Hg(II)-F/Hg(II)-U and MeHg-F/MeHg-U, these correlations increase somewhat with a minor change in the order of strength of weak influence: TDS, Cl, ALK, TEMP, and TP.

The LN transformation of these concentrations and ratios increase the correlations somewhat from very weak/weak to weak/moderate and DOC emerges as a moderate positive correlate with MeHg-U, MeHg-F and MeHg-F/MeHg-U, but only a very weak to weak positive correlate with Hg(II)-U, Hg(II)-F, or Hg(II)-F/Hg(II)/Hg(II)-U. This suggests either that DOC is facilitating MeHg transport to a greater extent than for Hg(II), which is borne out by the greater fraction of Hg(II) on particles than MeHg, on average, or that DOC is stimulating internal production of MeHg. DO emerges as a moderate to strong negative correlate with MeHg-U but not Hg(II), suggesting that it is related to internal production of MeHg. This topic is taken up in the next section. Confounding this observation is the apparent moderate positive influence on the ratio of MeHg-F/MeHg-U and the strong negative influence on MeHg-P/MeHg-U, suggesting perhaps that surface water redox potential controlled by DO is also affecting partitioning among particles, DOC, and the truly dissolved phase. It is also possible that DO is co-correlating with some other factor or constituent that has a greater influence on Hg(II) and MeHg partitioning. Nevertheless, one might infer that redox-sensitive aquatic species such as iron and/or manganese could mediate this phenomenon. Unfortunately, Fe monitoring ceased at ENR 004 when the ENR permit was issued, and Mn monitoring never occurred. There is some evidence that iron colloids mediate transport and/or transformations of Hg(II) and MeHg (Babiarz et al., 2001).

With the LN transformation, TP is now the "strongest" positive moderate to strong correlate with Hg(II)-U, while TSS and TN are weak to moderate positive correlates. There are no strong negative correlates. There are only very weak positive and negative correlations with Hg(II)-F/Hg(II)-U. While the weak positive correlation with TSS may seem contrary to expectation, since most of the TSS is only slowly settling or nonsettling organic matter, the more Hg(II) or MeHg that is sorbed to such solids, the farther these mercury species will be transported in the system before exchanging with stationary phase organic matter. TP may increase the internal production of such particles or be co-correlated with Hg(II) and MeHg loads, because they all originate primarily with the same EAA runoff.

Factors Influencing Inferred MeHg Production

The production of MeHg requires a bioavailable fraction of Hg(II) and the metabolic activity of methylating bacteria (Krabbenhoft et al., 2000; Krabbenhoft and Fink, 2001; Gilmour, **Appendix 2B-2**). Sulfate-reducing bacteria (SRB) are believed to be the primary methylators in the Everglades canals and marshes (Gilmour et al., 1998a,b; 1999). As summarized in **Table 2**, the absolute concentration of Hg(II) is weakly positively correlated with total dissolved solids (TDS), total phosphorus (TP), chloride (Cl), calcium (Ca), alkalinity (ALK), and temperature, in that order. The strongest weak correlation is with TDS, which are supplied by EAA runoff. This suggests that EAA runoff predominates in determining the loading of Hg(II) to the L-7 Canal. The correlation is too weak to have any predictive value, however.

The absolute concentration of MeHg-U could be an indicator of MeHg production in the EAA secondary canal system and/or the L-7 Canal. As discussed above, the ratio of U-MeHg/U-THg might be a better indicator of MeHg production in the EAA secondary canal system and/or the L-7 Canal. Inspection of the results in **Table 2** suggest that are no strong negative or positive correlations of any water quality parameter with either the absolute concentration of unfiltered MeHg (U-MeHg) or the ratio of MeHg-U/THg-U. The "strongest" weak correlations with MeHg-U are in the order Cl, TDS, ALK, and Ca, TP, and temperature. None of these correlations improve with MeHg-U/THg, but do improve noticeably with MeHg-F/THg-F and MeHg-P/THg-P, with some minor change in the order of "strongest" influence. The LN transformation again clarifies the strength of some these influences, with TP and DOC now becoming the "strongest"

moderate positive correlates and TEMP and SO4 now becoming the "strongest" weak positive correlate with MeHg-U. DO becomes the "strongest" moderate negative correlate. The direction and magnitudes of these influences would not be inconsistent with some, perhaps substantial internal production of MeHg in the L-7 Canal, primarily during the summer months. However, these correlations actually weaken when LN MeHg-U/THg-U and LN MeHg-P/THg-P are considered, but the positive correlation with DOC strengthens marginally for LN MeHg-F/THg-F.

The effect of sulfate on MeHg production is not obvious. Despite the fact that MeHg production is known to be carried out primarily by sulfate-reducing bacteria (SRB) and to be stimulated by the addition of sulfate to Everglades soils in laboratory microcosm and field mesocosm, there is only a weak positive correlation between the concentration of MeHg in water and the concentration of sulfate. The correlation does not improve substantially with MeHg-U/THg-U and only increases marginally with the LN transformation. This suggests that something else is limiting MeHg production most of the time. Perhaps this should not be surprising, because sulfate concentrations in EAA runoff and canal water are very high, averaging about 55 mg/L, as compared with south Florida rain, which averages < 1 mg/L (Guentzel, 1997). This is most likely also the case with N and P, which are also likely to be in substantial excess of the minimum physiological requirements of SRB at all times. This might be considered to be inconsistent with the moderate positive correlations between TP and MeHg-U, -F, and –P, but positive correlations with MeHg-U/THg-U weaken substantially, suggesting that the effect is on particle production and MeHg transport and not internal production.

Although the SRB also need a supply of short-chain carboxylic acids as a source of chemical energy for metabolism, growth, and reproduction, the correlation between MeHg-U with DOC is weakly positive and does not increase substantially with MeHg-U/THg-U. The correlations between DOC and MeHg-U and MeHg-F increase substantially with the LN transformation of these mercury species concentrations and with MeHg-F/THg-F, but not as much for the MeHg-U/THg-U. There is a moderate positive correlation with LN MeHg-F/MeHg-U and a moderate negative correlation with LN MeHg-P/MeHg-U. This also suggests that DOC's effect is on transport via its effect on partitioning and not internal production. The positive correlations of MeHg-U, -F, and -P and MeHg-U,-F, and -P/THg-U, -F, -P with Ca, Cl, and alkalinity could reflect their influences on Hg(II) uptake by SRB, but may also reflect their influences on the affinities for Hg(II) and MeHg for particle surfaces and DOC. pH, which has also been inferred to mediate Hg(II) uptake by methylating bacteria (Gilmour et al., 1991), is very weakly negatively correlated with MeHg-U and MeHg-U/THg-U. This correlation improves only marginally with the LN transformation.

Based on the preceding discussion, none of the water quality parameters is a good predictor of MeHg-U or MeHg/THg-U. While the correlations increase substantially for MeHg-F/THg-F and –P, they are still weak and must be considered to have no predictive value. The LN transformation improve this picture substantially, but no one factor or set of factors emerges with obvious predominance in its influence over MeHg-U/THg-U as a surrogate for internal MeHg production.

Factors Influencing Partitioning

Regarding the effect of water quality on partitioning of mercury species among dissolved and particle-bound phases, there are no strong positive or negative correlations between the untransformed mercury species concentrations or their ratios and any of the water quality parameters. TDS, Cl, ALK, and Ca appear to have the "strongest" weak influence on Hg(II) and MeHg partitioning among filtered and particulate phases. The use of the natural logarithmic transformation of the mercury species concentrations and their ratios changes this picture substantially but not significantly in the statistical sense. DOC, TEMP, ALK, and Ca are the "strongest" weak to moderate positive correlates with MeHg-F/MeHg-U, while DO, TSS, and pH are the "strongest" weak to moderate negative correlates. For Hg(II)-F/Hg(II)-U, there are no strong or moderate positive or negative correlations, with TEMP being the "strongest' of the weak positive correlates, and TSS and pH being the "strongest" of the weak negative correlates. With the LN transformation, the perspective changes dramatically. A moderate positive correlation with DOC and a weak negative correlation with TSS emerges with MeHg-F/MeHg-U and a moderate negative correlation with DOC and a weak positive correlation with MeHg-P/MeHg-U emerges. DO has a moderate negative influence and ALK, TEMP, and Ca have moderate positive influences on MeHg-F/MeHg-U. These same factors reverse the direction but not the magnitude of their influences for MeHg-P/MeHg-U. However, these strong anti-correlations between water quality parameters and MeHg-F/MeHg-U viz MeHg-P/MeHg-U may be an artifact of the way MeHg-P is calculated by subtracting MeHg-F from MeHg-U rather than measuring it directly. The preceding observations are consistent with competition between DOC and TSS for Hg(II) and MeHg, as mediated by such influential factors as pH, ALK, and Ca. These factors have also been identified as potentially significant moderators of partitioning or bioaccumulation in other empirical analyses of lake or wetlands data (Lange et al, 1993; Fink, 2001).

Factors Influencing MeHg Bioaccumulation

For the absolute value of the concentration of THg in mosquitofish, the "strongest" weak to moderate positive correlations are with DO and pH; virtually no correlation with TSS; and weak to moderate inverse correlations with TP, DOC, SO4, ALK, and Ca. The magnitudes of these correlations increase slightly with the LN transformation of the concentration of THg in mosquitofish and reach maxima with the ratio of the concentration THg in mosquitofish to the concentration of filtered MeHg (BCF/MeHg-F). Interestingly, however, the sign of the correlations with sulfate, chloride, and total nitrogen switch from negative to positive, suggesting that these apparent influences could, in part, be an artifact of dividing the mosquitofish THg concentration by the concentration of MeHg-F and their inverse correlations with MeHg-F. Since DO is moderately to strongly inversely correlated and TP moderately to strongly positively correlated with MeHg-U and MeHg-F, some of the apparent strength of the positive correlation with DO and the inverse correlation with TP could also result from the way the BCF is calculated. However, this cannot be the entire explanation.

On bioenergetics grounds, DO is expected to be inversely correlated with bioaccumulation (R. Harris, TetraTech, personal communication), because low DO requires that all aquatic organisms that breathe via gills must pass more water across their gills to meet their metabolic oxygen demand, and this, in turn, is expected to increase the MeHg uptake at the same time (Norstorm et al., 1976). For large fish, gill uptake of MeHg is trivial, but for small organisms this pathway is important (Rodgers, 1994). Because small organisms are eaten by large organisms, this effect is expected to propagate up the food chain. In fact, in the L-7 Canal, DO is positively, not negatively, correlated with BCF-U, -F, and –P. DO is also moderately positively correlated with pH, and pH is weakly to moderately positively correlated with MeHg bioaccumulation, so it

is possible that some of the apparent strength of the positive correlation between DO and MeHg bioaccumulation is being contributed by the positive influence of pH on MeHg bioaccumulation. DO is also moderately inversely correlated with Ca and DOC and weakly inversely correlated with sulfate and TP, and because each of these parameters is moderately to strongly inversely correlated with THg in fish and/or BCF-U, -F, and –P, some of the apparent strength of the DO correlation with BCF-U and BCF-F may be an artifact of these inverse co-correlations.

Surface water TP is moderately inversely correlated with the concentration of THg in mosquitofish, and the magnitude of the inverse correlation increases with BCF-U (**Figure 4**) and BCF-F, but weakens with BCF-P. The LN transformations generally strengthen these correlations. Now, if TP was exerting its influence on MeHg bioaccumulation by stimulating primary production (biodilution effect), one would expect that it would be strongly positively correlated with the concentration of MeHg-P and strongly inversely correlated with MeHg-F. In fact, the weak to moderate positive correlation with the concentration of MeHg-P in water is less than that for MeHg-U but does increase relative to MeHg-F. Moreover, Hg(II)-F is more weakly positively correlated with TP than is Hg(II)-U. However, the correlation with MeHg-F is positive, not negative. This could be because TP positively influences MeHg production to a greater extent than it influences the disposition of MeHg on particles through the biodilution effect. In fact, this effect has been demonstrated in a northern temperate lake mesocosm study (Rudd and Turner, 1983), but is absent in an Everglades mesocosm study (Gilmour, 2003). Moreover, the inverse correlation with BCF-P is weak, while the inverse correlation with BCF-F is moderate to strong. The fraction of THg that is MeHg actually shows a weaker correlation with TP than with the absolute concentrations of MeHg-U, -F, and –P.

DOC has been demonstrated to have a positive relationship with MeHg bioaccumulation in some aquatic systems (e.g., McMurtry et al., 1989; Sorenson et al., 1990) and an inverse relationship in others (e.g., **Figure 5**). This seemingly contradictory influence has been explained based on the difference in the hydrology of the lake. For lakes that receive the majority of their water from runoff, DOC in runoff carries Hg(II) that can be methylated *in situ*, as well as MeHg that has been produced *ex situ*. For lakes that receive the majority of their water via seepage, the DOC present in the lake competes with living and dead organic particles for Hg(II) and MeHg, thus decreasing their availability to the aquatic food chain and competes with the truly dissolved phase for MeHg, inhibiting its uptake organisms that absorb oxygen directly across their body surfaces or through gills.

No direct effect of sulfate on MeHg bioaccumulation was expected and only a weak to moderate positive correlation was observed (**Figure 6**), which could be a reflection of the weak negative correlation with MeHg-U and MeHg-U/THg-U. Although sulfide may mediate MeHg bioaccumulation at the sediment/water interface, and high sulfide may be correlated with high sulfate and low DO, sulfide was not monitored in surface water, where it is generally low, or in soil pore water, where it is generally much higher. While one can infer that sulfide will be high where MeHg is high, because both are byproducts of sulfate respiration by SRB, whether this co-correlation could explain some or all of the weak inverse relationship between surface water sulfate concentration and mosquitofish THg cannot be further evaluated without actual data.

The proper interpretation of the influence of TP on water MeHg and mosquitofish BCFs is complicated by its moderate to strong positive co-correlations with temperature, DOC (**Figure 7**), and sulfate (**Figure 8**) and its weak to moderate inverse co-correlation with DO. In particular, DOC has a strong affinity for Hg(II) (Haitzer et al., 2002) and MeHg (Amirbahman et al., 2002), so DOC competes with particle surfaces for both mercury species, resulting in a weakening of the partitioning of MeHg to particles, which, in turn, would be expected to weaken the influence of

TP on MeHg-F, MeHg-P, BCF-F, and BCF-P via biodilution. This is evident in the moderate correlation between DOC and the concentration of MeHg-F and the absence of a correlation with MeHg-P in the L-7 Canal data, so the apparent moderate positive correlation between water TP and MeHg-F or MeHg-P could be spurious. In addition, a moderate to strong positive correlation between DOC and the THg concentration in fish has been observed in data collected from hundreds of northern temperate lakes (Driscoll et al., 1994), but DOC has also been demonstrated to reduce significantly the biouptake of MeHg by fish from surface water in a controlled study (Chen et al., 1996). This latter effect is also evident in the L-7 Canal data, so the apparent moderate to strong inverse correlation of water TP with BCF-F could also be spurious as a consequence of co-correlation with DOC or vice versa.

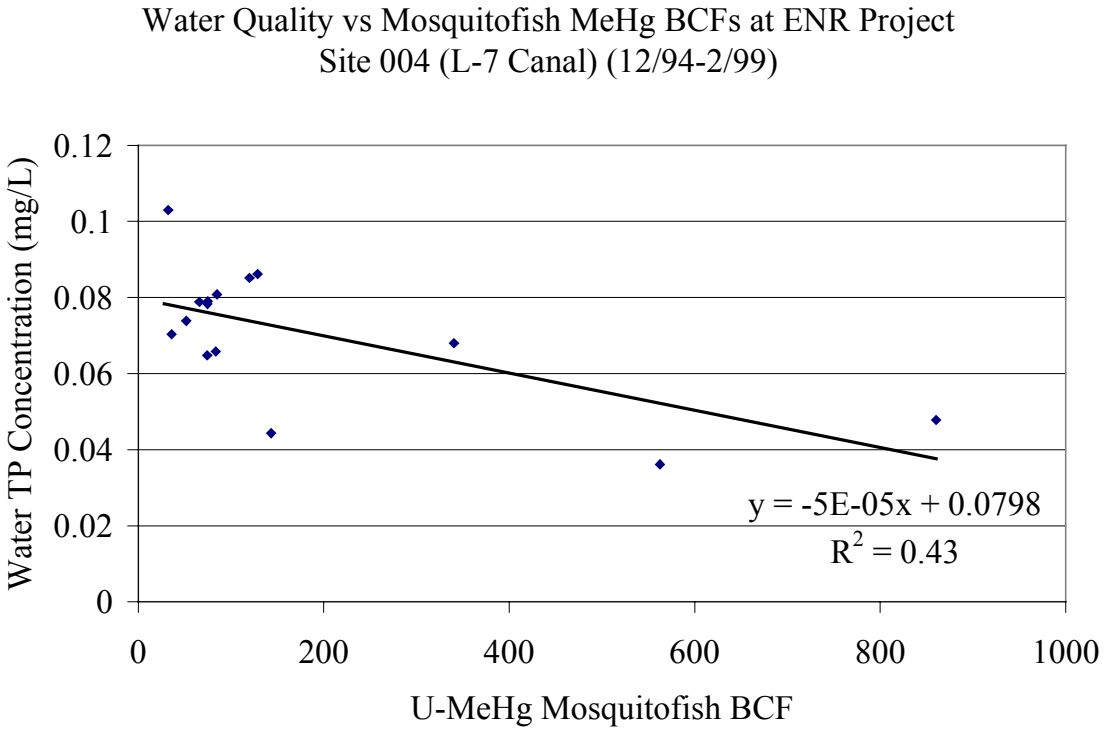

Figure 4. Water TP vs Mosquitofish U-MeHg BCF in the L-7 Canal at ENR 004

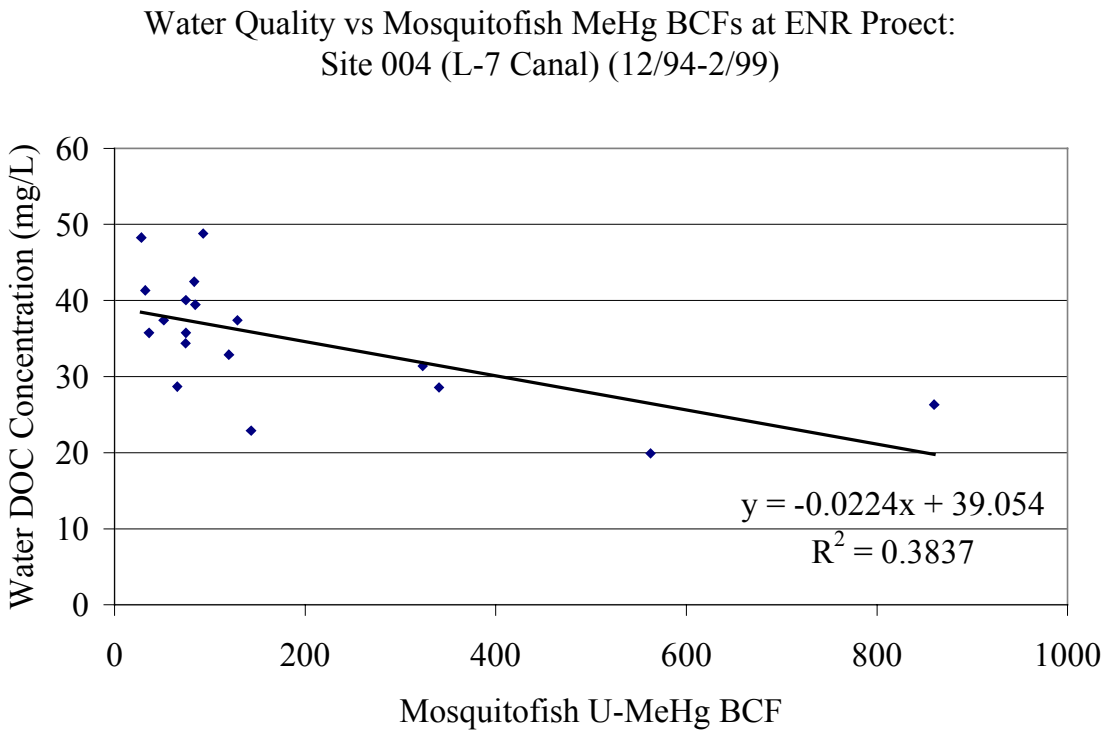

Figure 5. Water DOC vs Mosquitofish U-MeHg BCF in the L-7 Canal at ENR 004

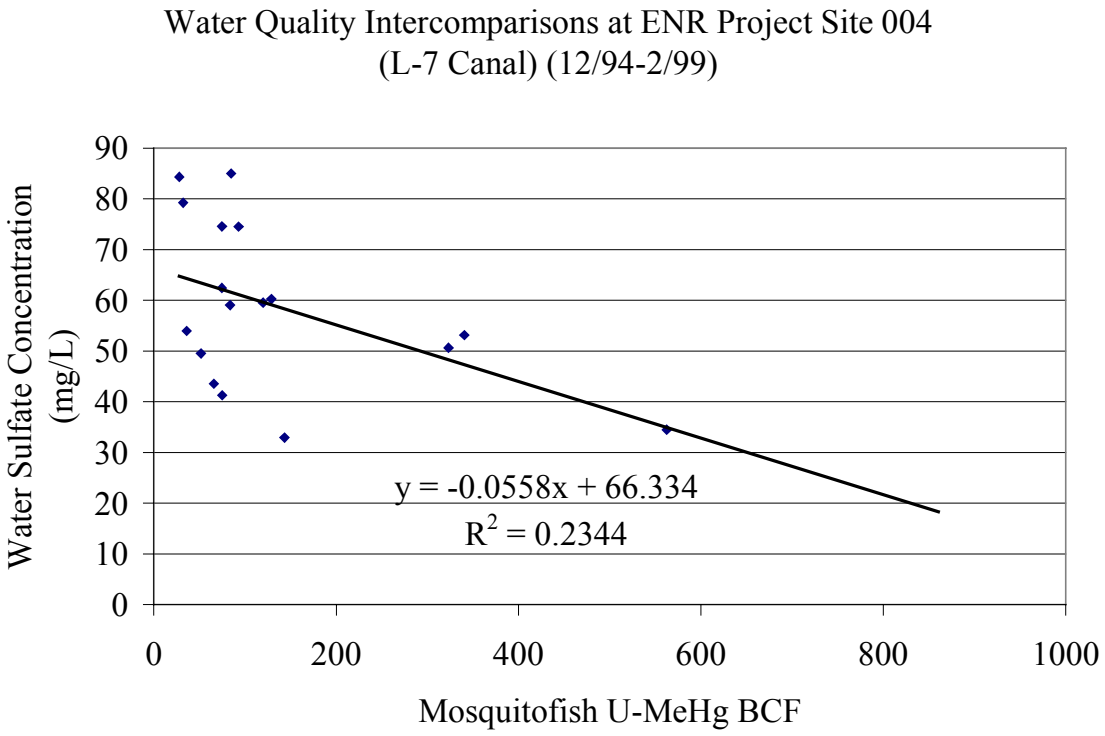

Figure 6. Water Sulfate vs Mosquitofish U-MeHg BCF in the L-7 Canal at ENR 004

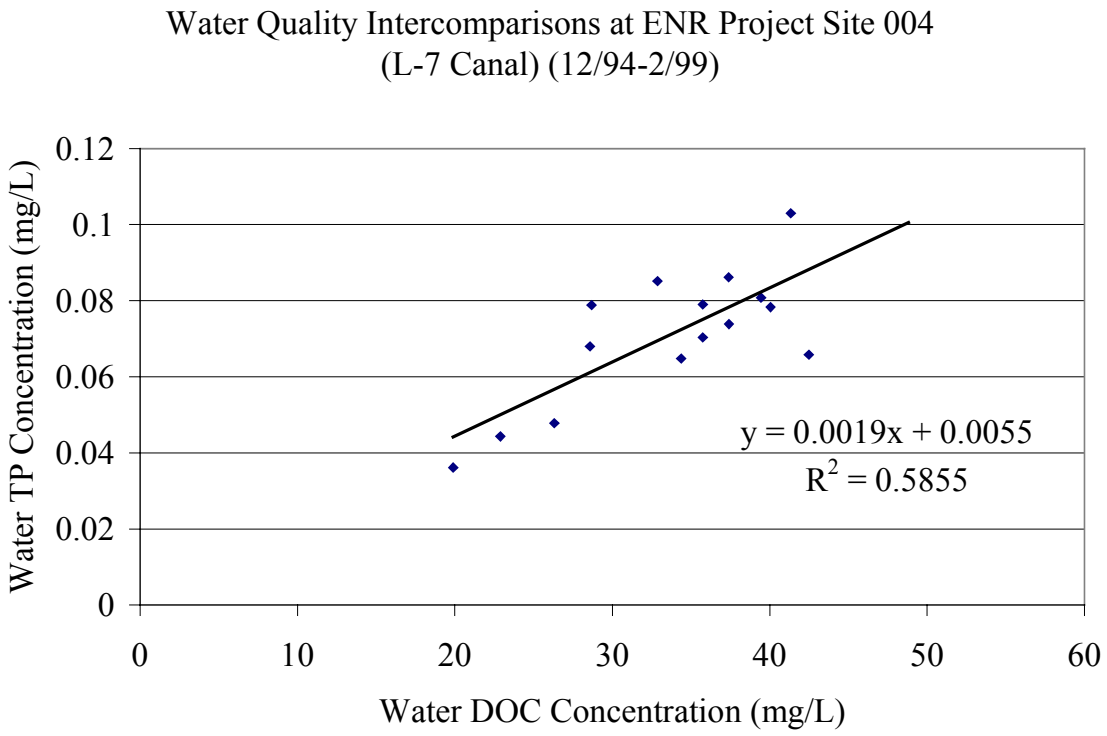

Figure 7. Co-variance of Water TP with Water DOC in the L-7 Canal at ENR 004

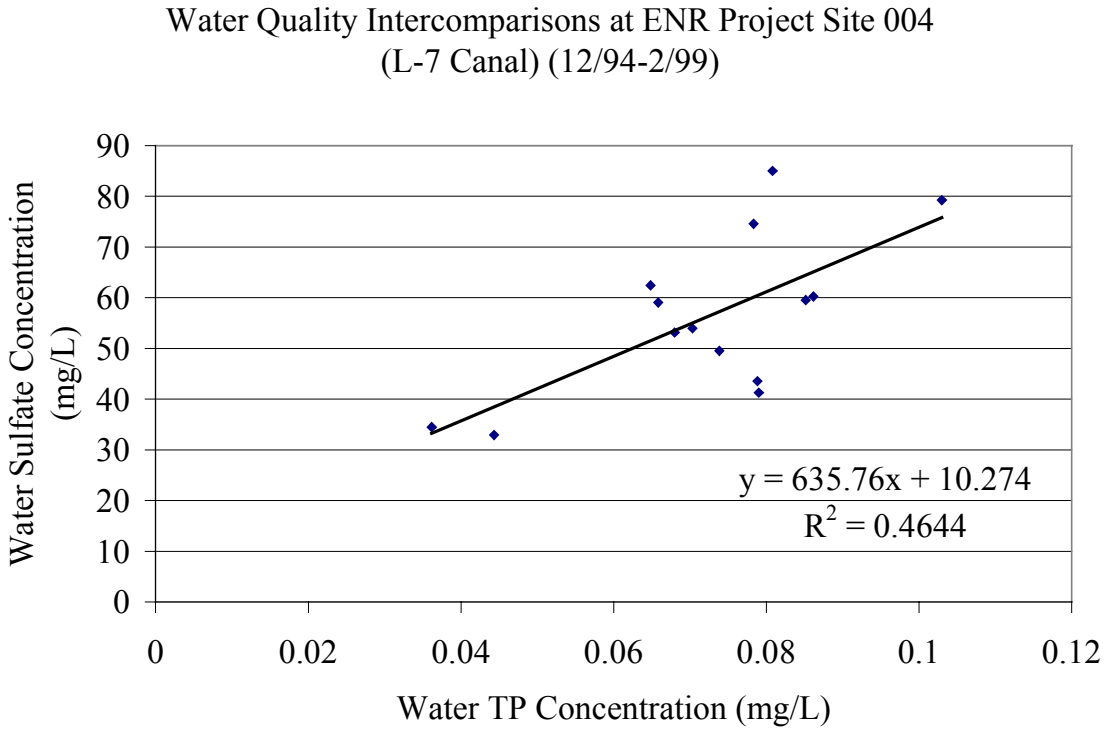

Figure 8. Co-variance of Water TP with Water Sulfate in the L-7 Canal at ENR 004

EXPLORATORY DATA ANALYSIS OF WATER QUALITY VS FISH THG: WCA-2A NUTRIENT GRADIENT

Background

The most intensively studied area in the Everglades is the nutrient-impacted zone downstream of the S-10 structures in the eastern lobe of Water Conservation Area-2A. In December 1993 the District began biweekly monitoring of surface water for a long list of constituents. Surface water sampling frequency was reduced to every other biweekly period in December 1996 based on a statistical analysis of the temporal redundancy in the data. Quarterly monitoring of pore water (5-20 cm) was initiated in August 1995, while semi-annual soil sampling began in January 1996 (0-5, 5-10, 10-30 cm cores). The soils and pore water monitoring studies were carried out in habitat dominated by cattail (C) and sawgrass (S). Soil sampling was reduced to annually beginning in the summer of 1997. Samples were collected along two transects: the "E" Transect, consisting of E1,E2, E3, E4, E5, and U1 and the "F" Transect, consisting of F1, F2, F3, F4, F5, and U3. Those sites are depicted in **Figure 9**. The study began in September 1997 with collections of mosquitofish composites at E1, F1, E4, F4, and U1 and U3. In August 1998 the study was modified to include sites F2, F3, and F5 and drop E1, E4, and U1 to better quantify the influence along one nutrient gradient. Sampling and analysis methods and procedures for surface water, pore water, and soil are described in Fink (2001). The parameters for which analyses were performed are summarized in **Table 6**.

A Summary of Previous Work

Following the approach taken by Lange et al. (1993) for Florida lakes, the District undertook an exploratory data analysis to determine which, if any, water quality constituents or environmental factors (e.g., water depth, temperature, distance from control structures) had the strongest influence on MeHg bioaccumulation in the Everglades. In the 1999 Everglades Interim Report, the District carried out a univariate and multivariate linear regression analysis of the relationships between THg in mosquitofish and water quality constituents for biweekly water quality data and quarterly mosquitofish THg data collected at WCA-2A study sites E1, F1, E4, F4, U1, and U3 (Rumbold and Fink, 1999). These sites are depicted in **Figure 9**. The water quality concentrations were averaged over the three months preceding the collection of the mosquitofish, based on an anecdotal maximum 90-day lifespan for the mosquitofish and a presumed seasonal response time for MeHg biomagnification of roughly one-quarter year. There was substantial covariance among the water quality constituents, limiting the robustness of the analysis.

Table 6. Summary of parameters analyzed in "F" transect surface water, pore water, and soil.

		SURFACE WATER (filtered)	PORE WATER (filtered) (5-20 cm)	SOIL (0-5 cm)
	PH	X	X	X
	FDO	X		
	SCON	X		
	TEMP	X		
WATER	DEPTH	X		
	ALK	X		
	AL			X
	CL	X	X	
	TC			X
	TOC			X
	DIC		X	
	DOC	X	X	
	CA	X	X	X
	CU	X		
	FE	X	X	X
	MG	X	X	X
	MN	X		
	K	X	X	X
	NA	X		
	ZN	X		
	NH3	X	X	
	NO2	X	X	
	NOX	X		
	NOXF	X	X	
	PO4	X		
	PO4F	X	X	
	SIO2	X		
	SO4	X	X	
	SULFIDE		X	
	TN			X
	TKN	X		
	TKNF	X	X	
	TP	X		X
	TPF	X	X	
	TIP			X
	TSS	X		
	REDOX		X	
BULK	DENSITY			X
	MOISTURE			X
	ASH			X
	AFDW			X
	OXAL			X
	OXFE			X
BICARB	SRP			X
BICARB	TP			X
HCL	SRP			X
KCL	SRP			X
NAOH	SRP			X
NAOH	TP			X

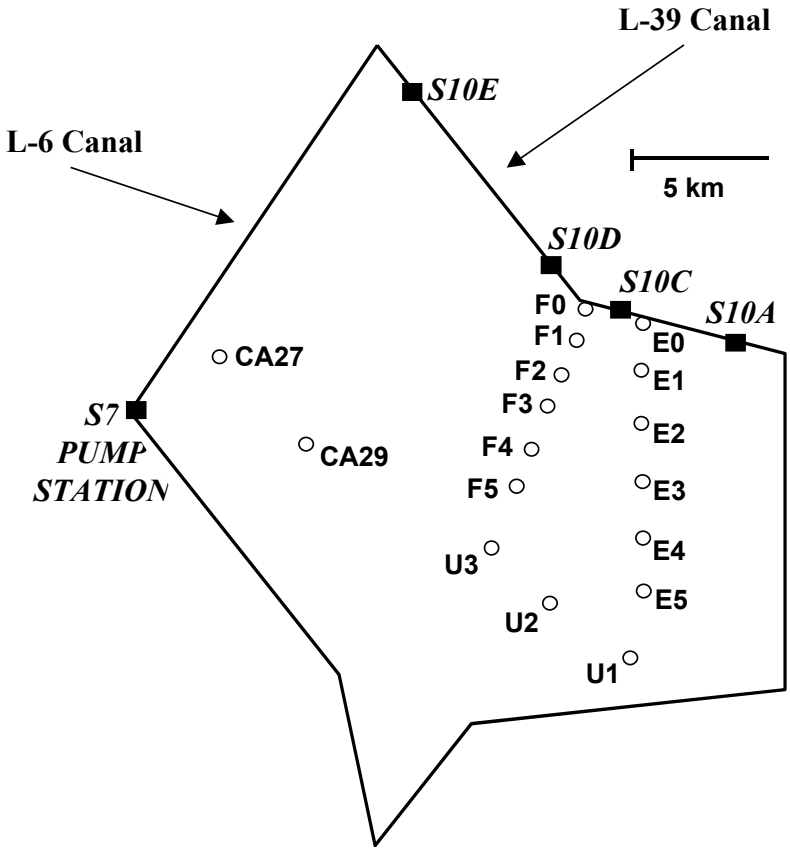

Figure 9. The "E" and "F" Transect research sites along a well-defined nutrient gradient in Water Conservation Area 2A

The results of that study produced the following one-variable nonlinear regression model with TP by analogy to that developed by Exponent (1998) and one- and two-variable linear regression models with the highest r^2 and lowest p values:

Regression: Mosquitofish THg (ug/Kg) = 1,150.7 x TP(ug/L) ^-1.34

Upper 95th percentile C.I.: Mosquitofish THg (ug/Kg) = EXP(7.89-1.99*[ln TP] + 0.18 [ln TP]^2)

Mosquitofish THg (ug/Kg) = 229.8 – (2.299 x Ca-F (mg/L))

$r^2 = 0.71$, $p < 0.001$, n = 24 data points at six sites

Mosquitofish THg (ug/Kg) = 163.89 + (4.74 x DOC (mg/L) – (3.66 x Ca-F (mg/L))

$r^2 = 0.83$, $p < 0.001$, n = 24 data points at six sites

These empirical models were unable to reproduce with acceptable accuracy the observed THg concentrations in mosquitofish collected at another well-studied interior marsh site further downstream of the research study sites in WCA-2A at WCA-3A-15 in the central Everglades. This was also true of the nonlinear phosphorus empirical model developed by Exponent (1998). The District concluded that such empirical models should not be used to predict the magnitude of MeHg bioaccumulation caused by changes in mercury loads and downstream water chemistry brought about by the operation of the STAs.

More Recent Work

This study has been prompted, in part, by an expected inverse correlation with surface water TP (PTI, 1994; PTI, 1995a,b; PTI, 1997; Exponent, 1998). However, in this exploratory data analysis, no hypotheses have been propounded or the likelihood of their acceptance or rejection evaluated using these data sets. Instead, the results are discussed in terms of consistency or inconsistency with the conceptual model developed above.

Since the publication of the original exploratory data analysis based on mosquitofish data collected through April 1999, additional data were collected on a quarterly basis through August 2000. The complete mosquitofish data set for the "F" Transect was then paired with the corresponding surface water, pore water, and soils data. In the correlation analysis phase, neither the mosquitofish THg data nor the surface water, pore water, or soils constituent concentration data were treated as the dependent variable. In the regression analysis phase, the mosquitofish THg concentration was treated as the dependent variable and the surface water, pore water, and soil constituent concentrations were treated as the independent variable. The correlation analysis phase was carried out first with all of the sites along the "F" Transect in aggregate and then with select individual sites. For surface water, each site was then analyzed individually. The analyses were repeated with the natural logarithmic (LN) transformation of the data. Pearson correlation coefficients for the paired data were calculated using the EXCEL ® spreadsheet program operating from a Windows 1997 platform.

Subsequently, it became apparent that the correlations were generally weak. It appeared that a more robust approach was required. In this approach, the THg mosquitofish data for the month of collection were paired with the water quality parameter data from the preceding month (t-1), t-2, t-3, etc. to t-6. The rational for this lagging scheme is that the physical, chemical, and biological conditions governing MeHg bioaccumulation in a mosquitofish at time t have already occurred

some time in the past. Since there is no way to know *a priori* what the cylcing, turnover, or response time of MeHg production or bioaccumulation is to a particular water, pore water, or soil parameter, it was assumed that the influences could extend back for as much as one-half year. The parameter values were then lag-averaged as t, t-1; t, t-1, and t-2; t-1, t-2, and t-3; t-2, t-3, and t-4; t-4, t-5, and t-6. The rationale for the averaging scheme is that the mosquitofish are responding to MeHg mass in prey that was produced several days, weeks, or months previously and that the average life expectancy of a mid-size mosquitofish is on the order of 90 days.

The above-described scheme was then applied to the following combinations of surface water data: untransformed mosquitofish THg X untransformed water, pore water, and soil parameters; untransformed X log-transformed; log-transformed X untransformed; and log-transformed X log-transformed for all six sites along the "F" Transect. There were too few data pairs to allow a multivariate regression analysis for individual sites. All of the univariate and multivariate linear regression analyses were carried out by SAS. However, during the multivariate runs, it quickly became apparent that when all of the surface water parameters were included, the multivariate model was underconstrained. This required the deletion of a number of parameters from further consideration. The deletion criteria were based on the weakness of the univariate correlation analysis in the scoping study (low r value) or redundancy (e.g., TKN and TN). The final list of parameters for the multivariate regression analysis of surface water were: ALK, DOC, SO4, Cl, SiO2, TP, TN, and Ca .[Unfortunately, all of these analyses have not been completed as of this writing. The results generated to date will be supplied to the reviewer upon request.]

For sediment pore water, it was assumed that this reservoir was averaging the influences of surface water parameters on MeHg production and bioaccumulation on the order of seasonally, which is the same as for the mosquitofish, or about 90 days. While a three-month lag analysis would be possible in theory, in practice the irregular sampling schedule resulted in very few appropriately aligned data pairs. For soils, the sampling frequency was reduced to annually well before the initiation of the mosquitofish collections along the "F" Transect, so there was no opportunity to carry out a lag analysis with a resolution less than annually. Therefore, the exploratory correlation analysis was carried out on sediment pore water and soils data collected in month t with the contemporaneous mosquitofish THg data. There are too few data sets to carry out a multivariate linear regression analysis for even the reduced data set derived for the surface water analysis.

Results and Discussion

The results of the exploratory correlation analysis for surface water (average of t-1, t-2, and t-3), pore water, and soil data are summarized in **Table 7** for the aggregate set of data for all five study sites along the "F" Transect. The results indicate that pore water chemistry has the strongest correlations with mosquitofish THg. The strongest positive LN-transformed correlates between mosquitofish THg and pore water are in the range $0.5 < r < 0.75$ in the order: Fe, SO4, K. The strongest negative correlates are in the range $-0.5\ \ r < -0.75$ in the order: Mg, Cl, Ca, DOC, PO4, sulfide. The relationship between pore water iron, DOC, TP-F, SO4, or $S^{=}$ and MeHg bioaccumulation in mosquitofish is depicted in **Figures 10**, **11**, **12**, **13**, and **14**, respectively. High pore water Fe concentrations could be associated with changes in redox that favor Hg(II) release from refractory to more labile inorganic complexes and/or the oxidation of sulfide to sulfate, with a concomitant shift in pore water chemistry to favor the formation of Hg(II) complexes that are more readily taken up by methylating bacteria, primarily sulfate-reducing bacteria (SRB). The weak but positive correlation with pore water sulfate would not be inconsistent with this conjecture.

Table 7. Summary of Pearson correlation analysis between mosquitofish THg and surface water, pore water, and soil chemistries

		SURFACE WATER (filtered)	LN TRANS	PORE WATER (filtered) (5-20 cm)	LN TRANS	SOIL (0-5 cm)	LN TRANS
	PH	0.2480483		-0.229			
	FDO	0.4625906	0.350768				
	SCON	-0.263	-0.112				
	TEMP	0.215	0.051				
WATER	DEPTH	-0.059	-0.038				
	ALK	-0.29	-0.247				
	AL					-0.043	0.1
	CL	-0.158	-0.107	-0.402	-0.626		
	TC					-0.074	-0.087
	DOC	-0.142	-0.041	-0.349	-0.5712165		
	CA	-0.316	-0.155	-0.411	-0.618	-0.19	-0.245
	CU	-0.282	-0.208				
	FE	0.07	0.077	0.372	0.62	0.147	0.078
	MG	-0.171	-0.055	-0.418	-0.631	-0.2	0
	K	0.174	0.273	0.289	0.2125	0.504	0.627
	NA	-0.14	-0.085				
	ZN	-0.112	-0.102				
	NH3	0.142	0.069	0.396	-0.036		
	NOXF	-0.276	-0.171	0.117	-0.017		
	PO4F	0.465	0.301	-0.109	-0.376		
	SIO2	-0.302	-0.223				
	SO4	0.139	0.409	0.144	0.236		
	SULFIDE			-0.134	-0.169		
	TN					0.487	0.281
	TKN	-0.254	-0.269				
	TKNF	-0.035	0.035	0	-0.352		
	TP	0.009	-0.207			-0.189	-0.462
	TPF	0.052	-0.13	-0.095	-0.386		
BULK	DENSITY					-0.02	-0.248
DRY	WEIGHT					-0.096	-0.142
	ASH					-0.11	-0.076
	OXAL					-0.09	-0.016
	OXFE					0.297	0.125

The strongest negative pore water constituent correlates are species that are known to complex or mediate the complexation of Hg(II), which could affect its bioavailability to SRB, or of MeHg, which could affect its bioavailability to demethylating bacteria. The relatively weak inverse relationship with pore water sulfide is much less than that observed in the USGS ACME data collected from ten sites across the Everglades in the period 1995-1999 (Fink, 2002). However, there the pore water sulfide was collected from 0-5 cm where MeHg production is believed to be a maximum (Gilmour et al., 1998b), whereas the "F" Transect pore water is

collected from 5-20 cm depth. This would have a tendency to wash out the responsiveness of the pore water to sulfide production and destruction, as well as strong statistical evidence of its influence on Hg(II) bioavailability to SRB, as has been hypothesized by others (Gilmour, 1998a,b; 1999).

Unexpected weak to moderate positive correlations were observed between soil K or TN and mosquitofish THg. This may reflect shifts in the microbial communities and their physiological requirements as one proceeds down the nutrient gradient. However, these correlations could also arise from spurious associations unrelated to any cause-effect relationship. A moderate inverse relationship between soil TP and mosquitofish THg also emerges from the analysis that is much stronger than the corresponding surface water correlation (**Figure 15**). This may be a result of the soil TP concentrations more accurately reflecting the annual average trophic state of the aquatic environment than the instantaneous concentration in the water column, which could be of the same order as the response/integration time of the mosquitofish population to net MeHg production and bioaccumulation, taking into account seasonal trophic dynamics (P. Rawlik, personal communication). However, much shorter response/integration times have been observed for the mosquitofish in other settings (e.g., on the order of 14- to 28-days per Rawlik, et al., 2001a,b). Unfortunately, neither total sulfur nor acid volatile sulfide was measured in soil along the "F" Transect, and the latter has been demonstrated to be inversely correlated with the percent MeHg in surficial soil as a surrogate for the net production rate, so the potential for positive or inverse co-correlations between TP or TN and TS or AVS cannot be ruled out. More unfortunately, THg and MeHg in soil were not measured concurrent with mosquitofish collection, so there is no way to relate mosquitofish THg to percent MeHg in surficial soils as a surrogate for net MeHg production.

A potential inconsistency in the results need to be noted and discussed. There is virtually no correlation between surface water TP and mosquitofish THg in the untransformed data and weakly negative in the LN-transformed data, while OPO4 exhibits a weak to moderate positive correlation for the untransformed data ($r = 0.46$) that weakens somewhat with the LN-transformation ($r = 0.31$). However, a closer inspection of the OPO4 data set indicates that a number of data were censored due to fatal flags, substantially reducing the number of data points ($n = 24$) relative to other parameters (e.g., $n = 44$ for TP). This could result in a magnitude of correlation that does not accurately reflect the magnitude of pore water OPO4 influences on SRB activity or Hg(II) bioavailability. However, it is less likely that the sign of the correlation would change. If OPO4 were acting on the magnitude of MeHg bioaccumulation via the process of biodilution, then an inverse, not positive, correlation with mosquitofish THg would have been expected. It is possible that the same processes that liberate Fe and create conditions conducive to the stimulation of mercury methylation may also liberate bound OPO4, resulting in an apparent correlation with MeHg production and bioaccumulation that is a result of co-correlation and not mechanistic influence on the MeHg production rate *per se*.

Table 8 displays the linear regression model with the highest r^2 value for log transformed mosquitofish THg vs the log-transformed surface water parameter subset for lags 0 through 6 months. TP has a negative coefficient for all lags, but the other parameters exhibit various patterns of switching from positive to negative slope, suggesting that their influences are on different processes with different cycling/response times. A more detailed analysis must await the completion of the entire exploratory scheme.

In the preceding analysis, the sites along the nutrient gradient were aggregated. However, if the concentration of TP in surface water TP is a robust predictor of MeHg bioaccumulation in fish via the mechanism of biodilution, then the correlations that emerge from the exploratory data

analysis in aggregate should also be reflected at the individual sites. To test the hypothesis that the correlations in space are reflected in correlations in time, the correlations for F1, F4, and U3 were also evaluated individually. These sites were chosen because they span the length of the transect and have the longest individual periods of record. Again the focus is on the biodilution hypothesis mediated primarily by water column TP. Here the average of the parameter values for months t-1, t-2, and t-3 were paired with the mosquitofish data from month t. The lag three month average was chosen because it yielded the strongest correlation with TP in the preceding phase of the study. The plot of the LN-transformed surface water TP vs mosquitofish THg for the aggregate sites is **Figure 16**. The plots of LN-transformed mosquitofish THg vs surface water TP at F1 only with (**Figure 17**) and then without (**Figure 18**) the post-dryout anomalous data points and at U3 (**Figure 19**) make it clear that the weak negative correlation for the aggregate sites disappears for the individual sites with the longest periods of record. In fact, the slope at F1 is distinctly positive, yet it is at F1, where TP is highest and plant production is highest, that the biodilution effect should be most readily detected.

The preceding results suggest that something other than TP is the predominant influence on MeHg bioaccumulation at sites along the WCA-2A "F" Transect. Why this might be the case is taken up in a later section that reiterates a previously published biodilution calculation for THg at F1 and U3 (Fink and Rawlik, 2000).

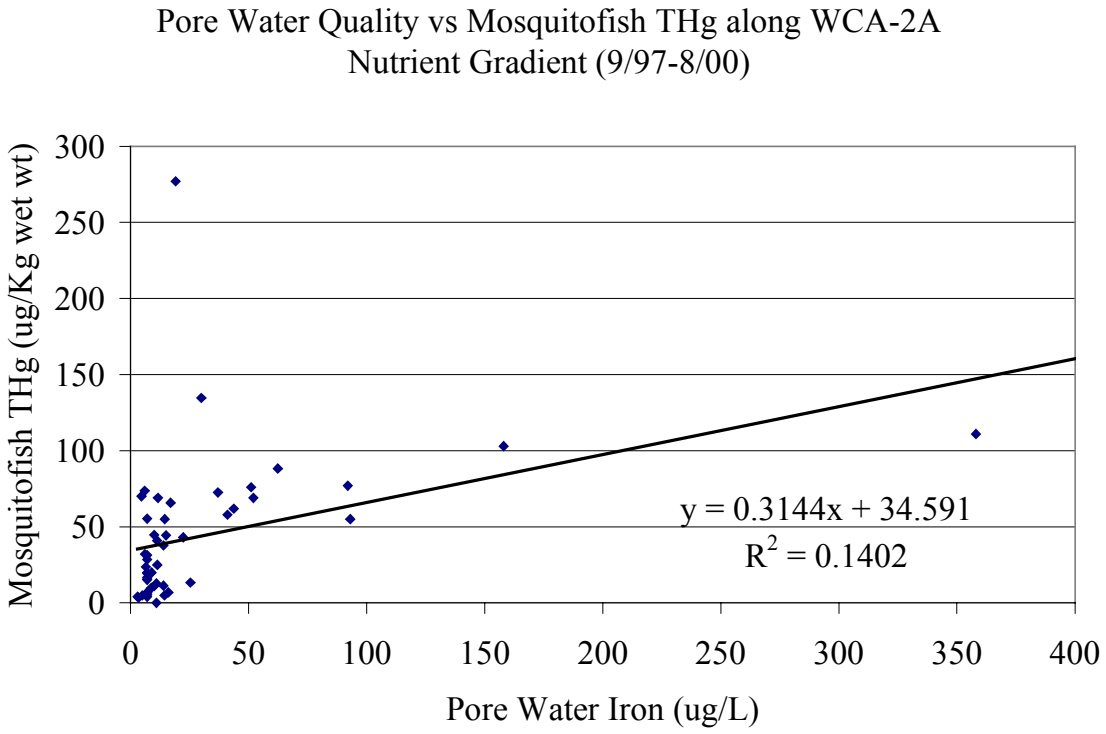

Figure 10. Mosquitofish THg (average of homogenized composite) vs filtered pore water iron in a depth-integrated sample from 5-20 cm collected at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

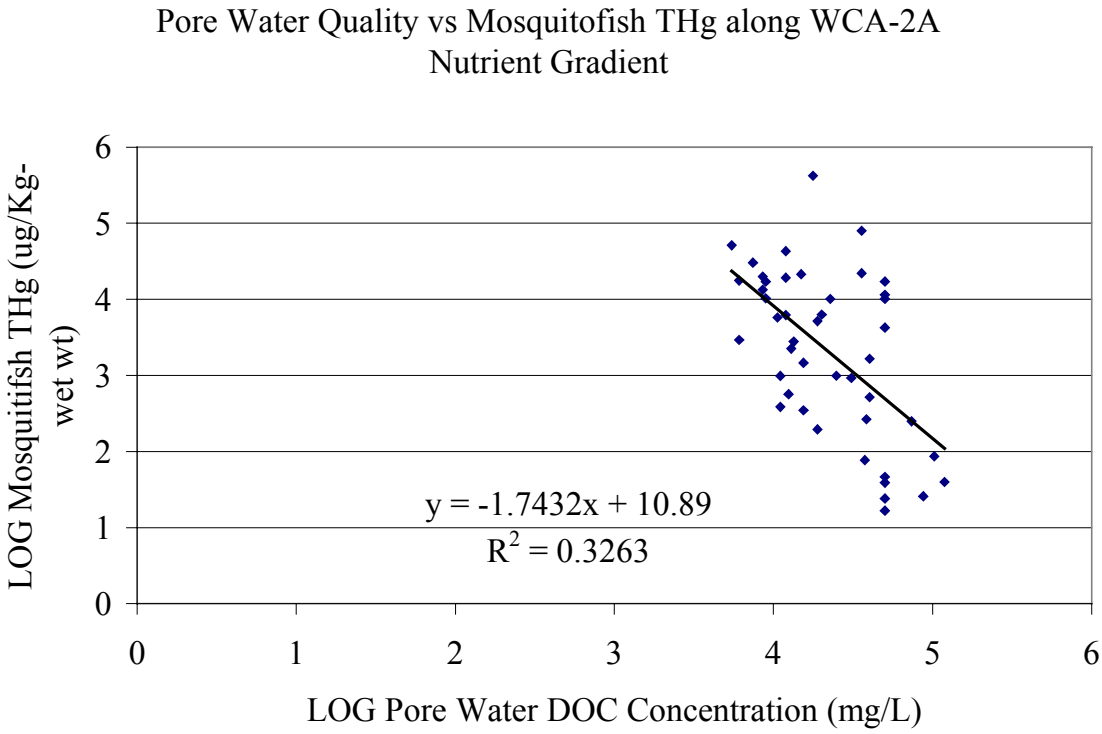

Figure 11. Mosquitofish THg (average of homogenized composite) vs filtered pore water dissolved organic carbon (DOC) in a depth-integrated sample from 5-20 cm collected at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

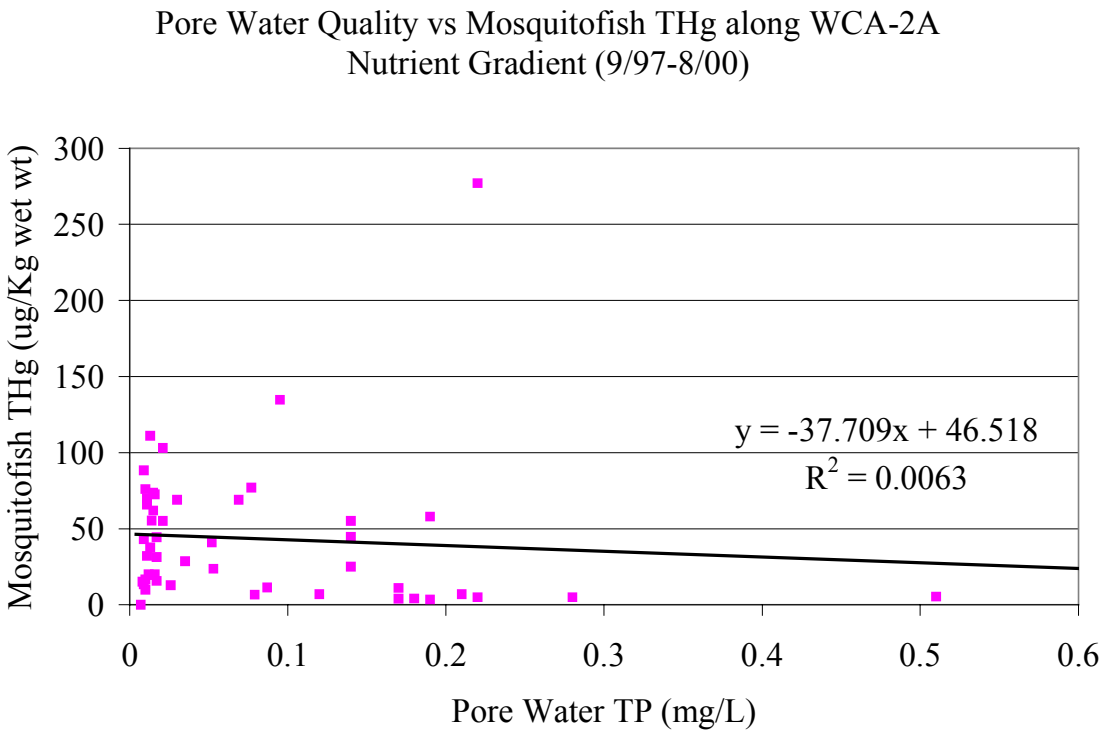

Figure 12. Mosquitofish THg (average of homogenized composite) vs filtered pore water total phosphorus (TP) in a depth-integrated sample from 5-20 cm collected at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

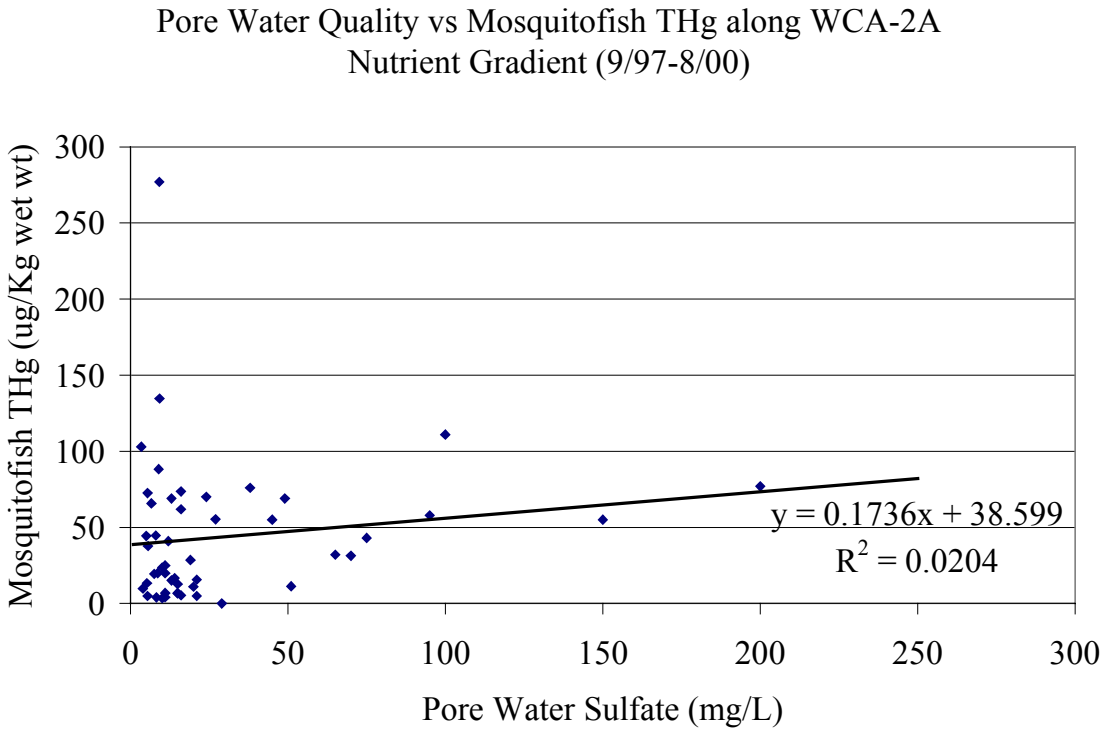

Figure 13. Mosquitofish THg (average of homogenized composite) vs filtered pore water sulfate in a depth-integrated sample from 5-20 cm collected at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

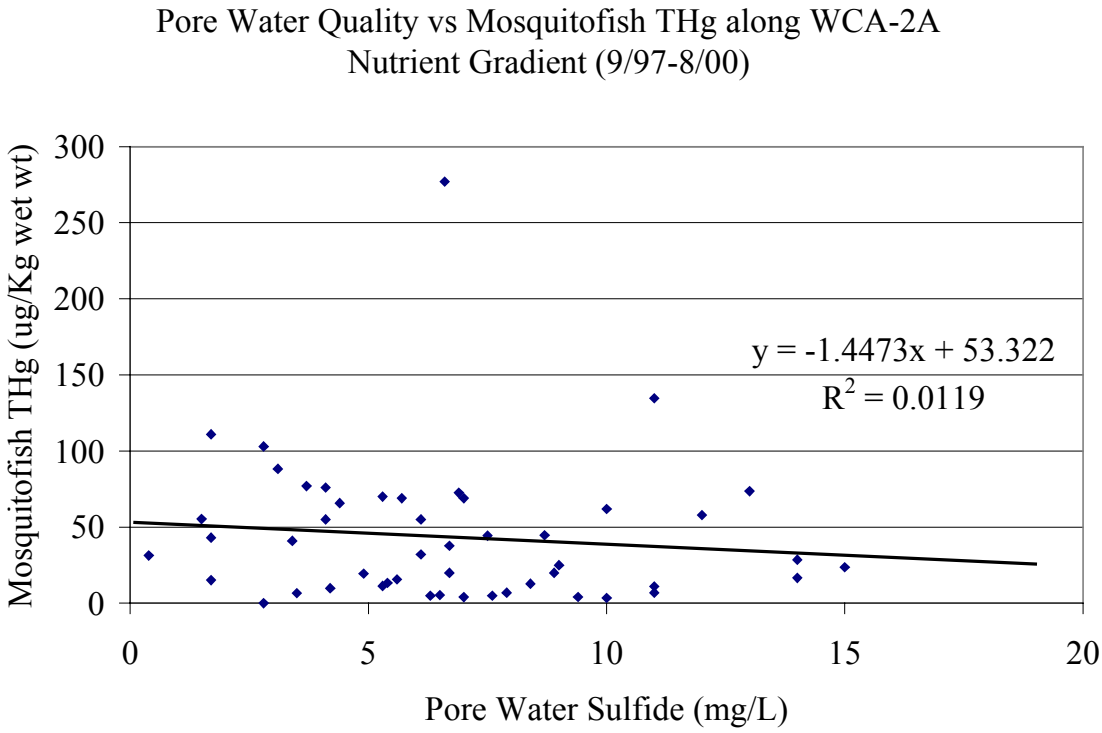

Figure 14. Mosquitofish THg (average of homogenized composite) vs filtered pore water sulfide in a depth-integrated sample from 5-20 cm collected at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

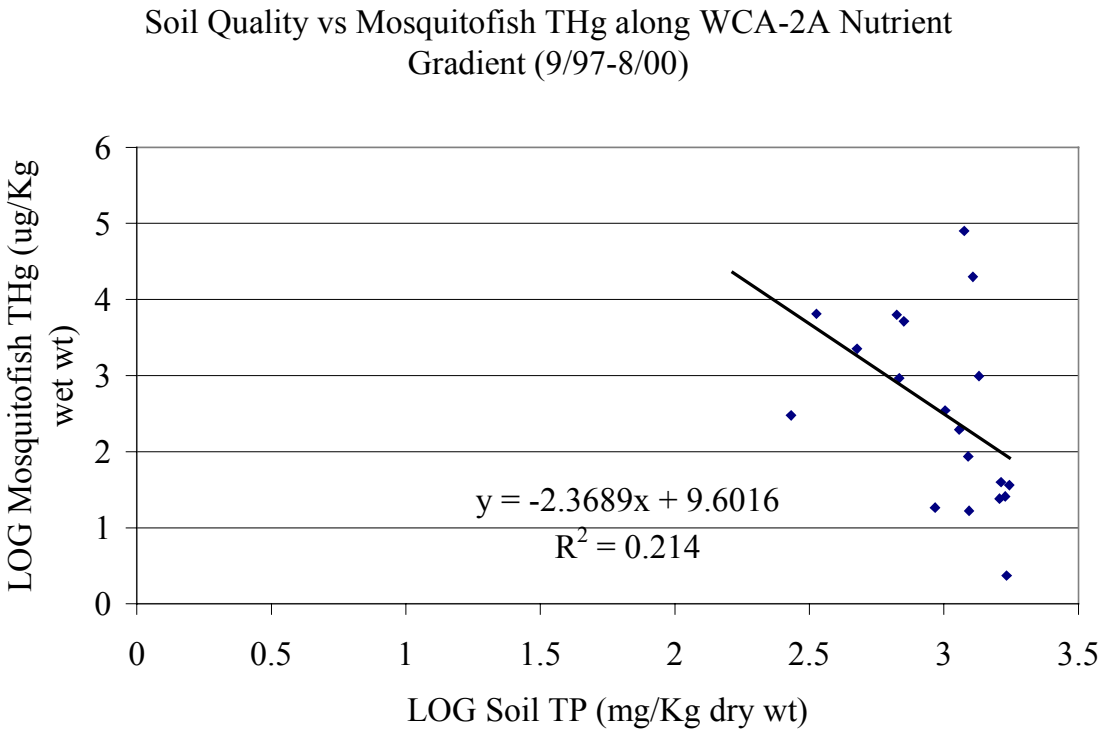

Figure 15. Mosquitofish THg (average of homogenized composite) vs soil total phosphorus (TP) in a depth-integrated sample from 0-5 cm collected in a sawgrass habitat at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

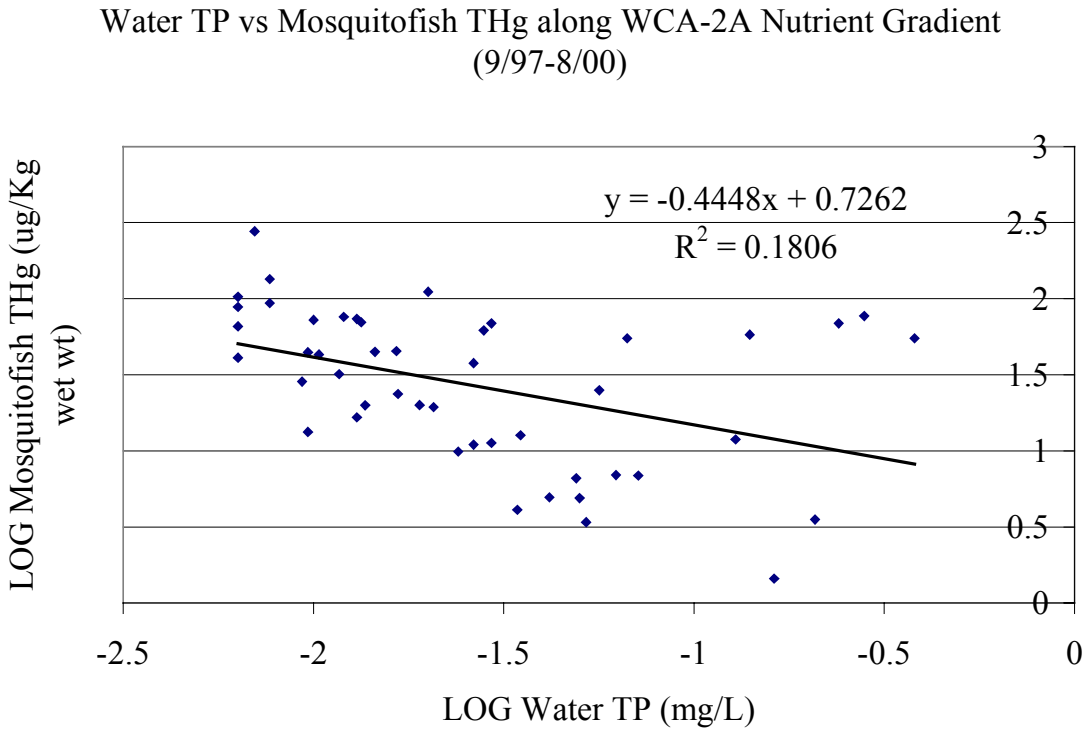

Figure 16. Mosquitofish THg (average of homogenized composite) vs surface water total phosphorus collected by subsurface manual grab at six sites along a well-studied nutrient gradient in WCA-2A on a quarterly basis from 9/97 through 8/00.

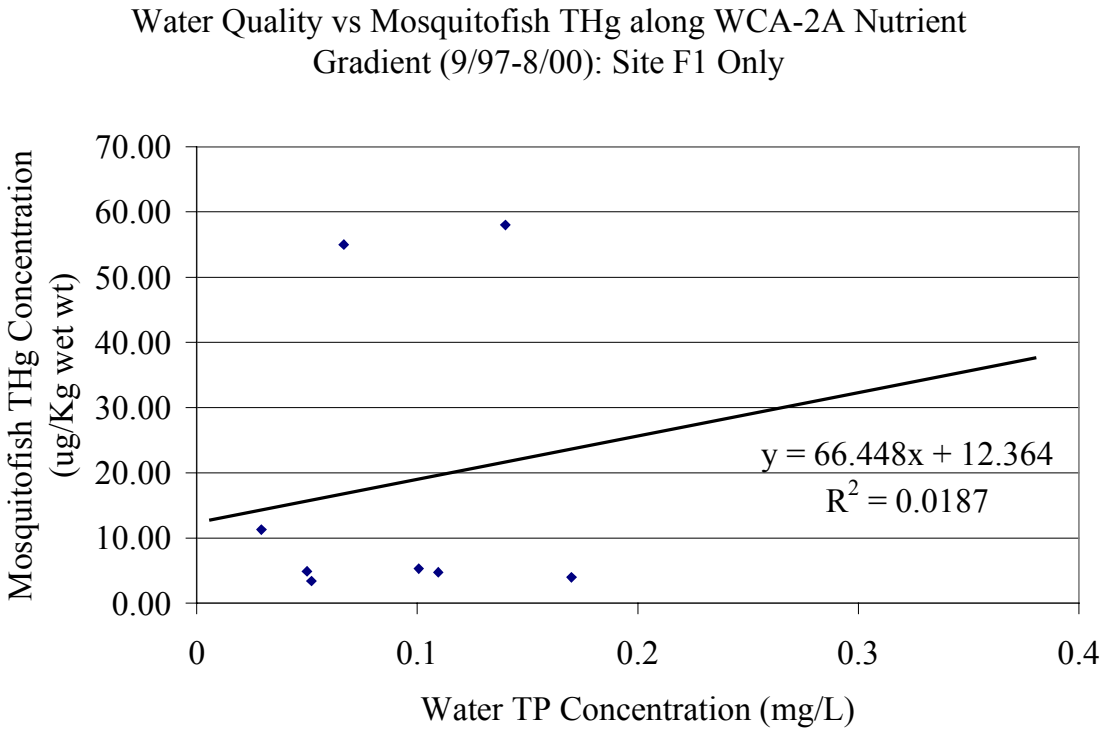

Figure 17. Mosquitofish THg (average of homogenized composite) vs surface water total phosphorus collected by subsurface manual grab only at WCA-2A-F1, the most eutrophic site, on a quarterly basis from 9/97 through 8/00.

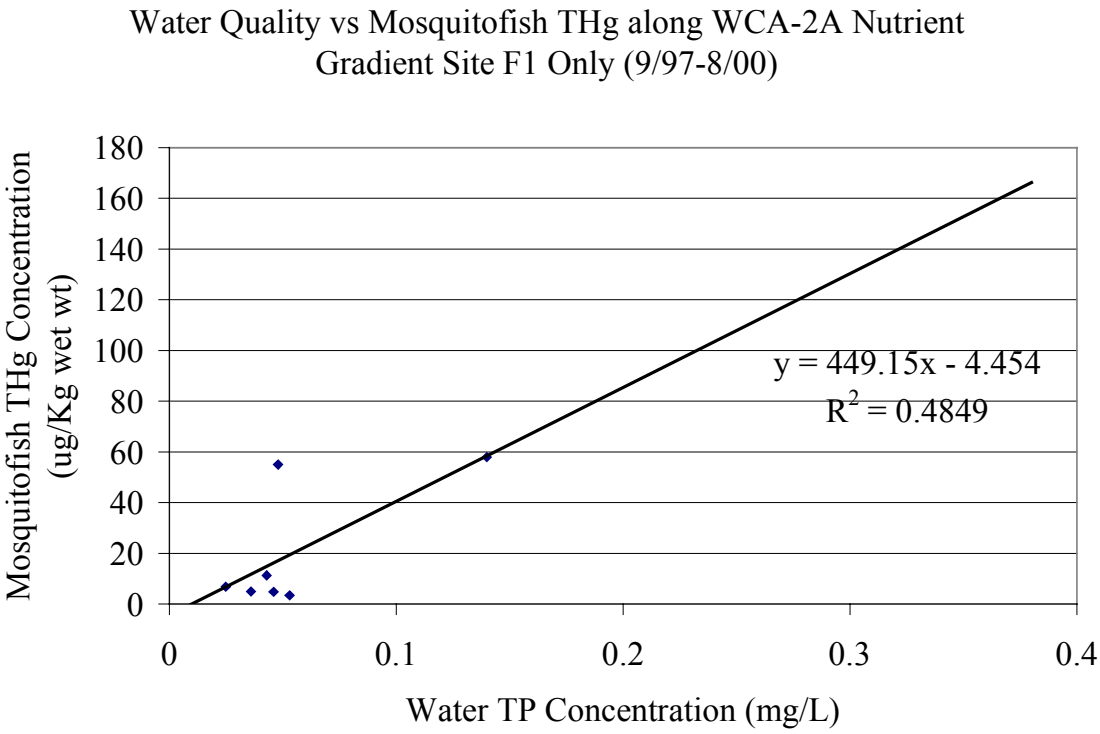

Figure 18. Mosquitofish THg vs Water TP using data only from Site WCA-2A-F1, the most eutrophic site, deleting the two data points collected following extended periods of drawdown and dryout

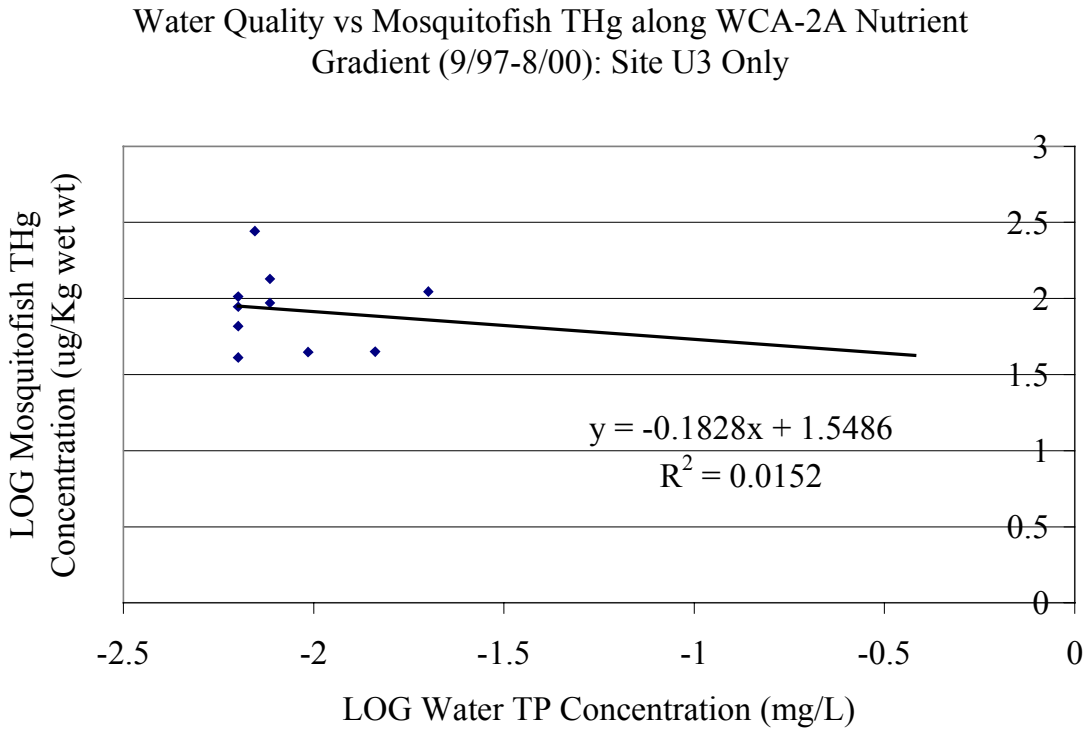

Figure 19. Mosquitofish THg (average of homogenized composite) vs surface water total phosphorus collected by subsurface manual grab only at WCA-2A-U3, the most oligotrophic site, on a quarterly basis from 9/97 through 8/00.

EXPLORATORY DATA ANALYSIS OF WATER QUALITY VS FISH THG: ANNUAL PERMIT COMPLIANCE MONITORING SITES

Largemouth bass (up to n = 20), sunfish (up to n = 20), and mosquitofish (homogenized composite of 75-250 fish subsampled n = 5 times) have been collected annually at 10 interior Everglades monitoring sites beginning in the fall of 1998. Due to habitat and access limitations, the contractor defaults to the nearest area where fish can be found, which is often the nearest canal. As a consequence, of the 10 sites, only at the interior site in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LOX 4), the reference site in WCA-2A (WCA-2A-U3), the Everglades "hot spot" in WCA-3A (WCA-3A-15), and the site at the end of the stub canal in the Everglades National Park (P33) are largemouth bass, sunfish, and mosquitofish routinely obtained from true interior marsh sites. Water quality samples were also collected from these sites monthly by the District as part of general system and/or permit-mandated monitoring. The annual averages were calculated for 1998, 1999, 2000, and 2001 and paired with the corresponding fish data for univariate linear regression analysis. The data were then log transformed and the analysis was repeated. The Pearson correlation coefficient, r, and the number of data pairs, n, for each type of fish and constituent are summarized in **Table 9.** Co-variance with water column constituents with high "r" values was tested to evaluate the robustness of the correlations. For largemouth bass, the strongest correlations were with water total organic carbon, color, dissolved organic carbon, total dissolved solids, total phosphorus, total Kjeldahl nitrogen, and sulfate, in that order. Sulfate exhibited a weak co-variance with TP, while the TP co-variances with TOC, DOC , and water color were relatively high. Whether the annual average TP concentration is the cause of the inverse relationship with mosquitofish, sunfish, and largemouth bass, one or more of its co-variates is the cause, or the correlation is only a spurious association cannot be ascertained with the available information.

Table 9. Linear Correlation Analysis Results for Permit Compliance Monitoring Sites: Fish THg vs Water Quality Parameters

	Largemouth bass	Sunfish	Mosquito-fish
	(n=14)	(n=12)	(n=17)
TEMP	0.20143	0.049489	0.214818
DO	-0.06051	-0.24575	-0.27228
pH	-0.10356	-0.00568	0.210425
TSS	-0.27017	-0.17437	-0.3208
TKN	-0.63397	-0.66937	-0.62245
TP	-0.65609	-0.62812	-0.13712
CA	-0.09609	- 0.05463	0.046989
CL	-0.51798	- 0.53154	-0.60303
SO4	-0.64114	-0.70373	-0.59979
ALK	-0.32255	-0.53998	-0.33835
DOC	-0.71949	-0.76361	-0.73733
TDS	-0.68423	-0.66357	-0.5754

The concentrations of THg in mosquitofish, sunfish, and bass fillets are plotted against surface water DOC in **Figures 20**, **21**, and **22**; surface water SO4 in Figures **23**, **24**, and **25**, and surface water TP in **Figures 26**, **27**, and **28**. The co-correlation between surface water TP and DOC and TP is graphed in **Figure 29**.

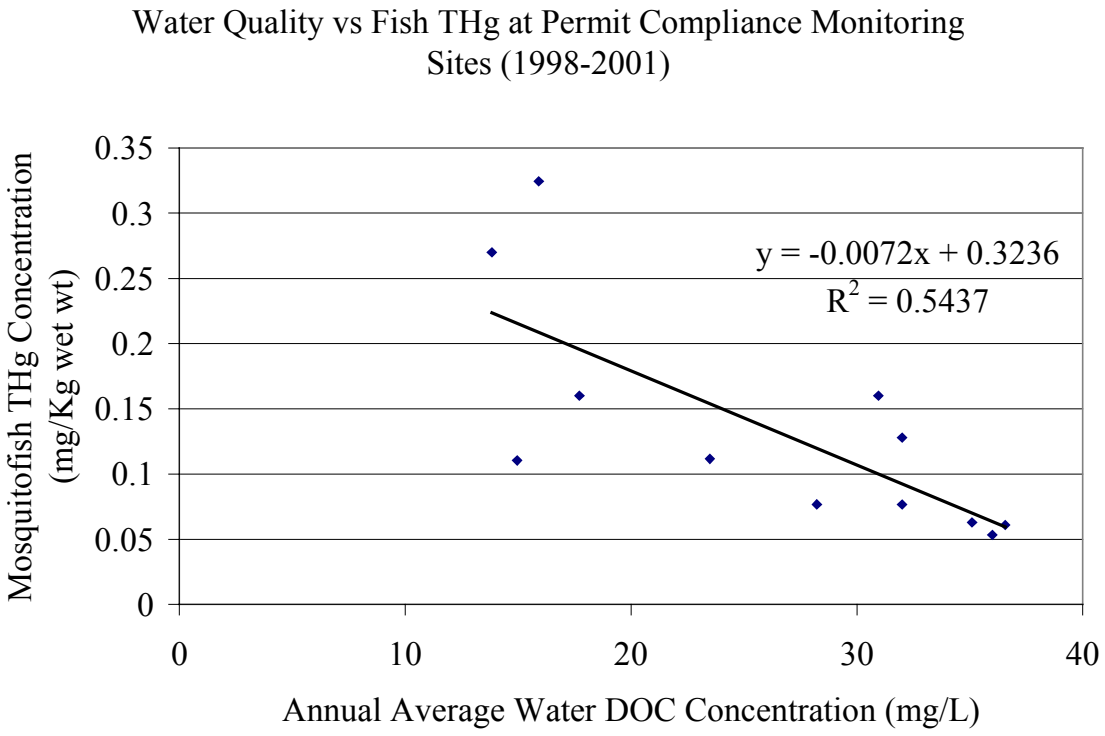

Figure 19. Mosquitofish THg concentration vs annual average water DOC concentration at permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

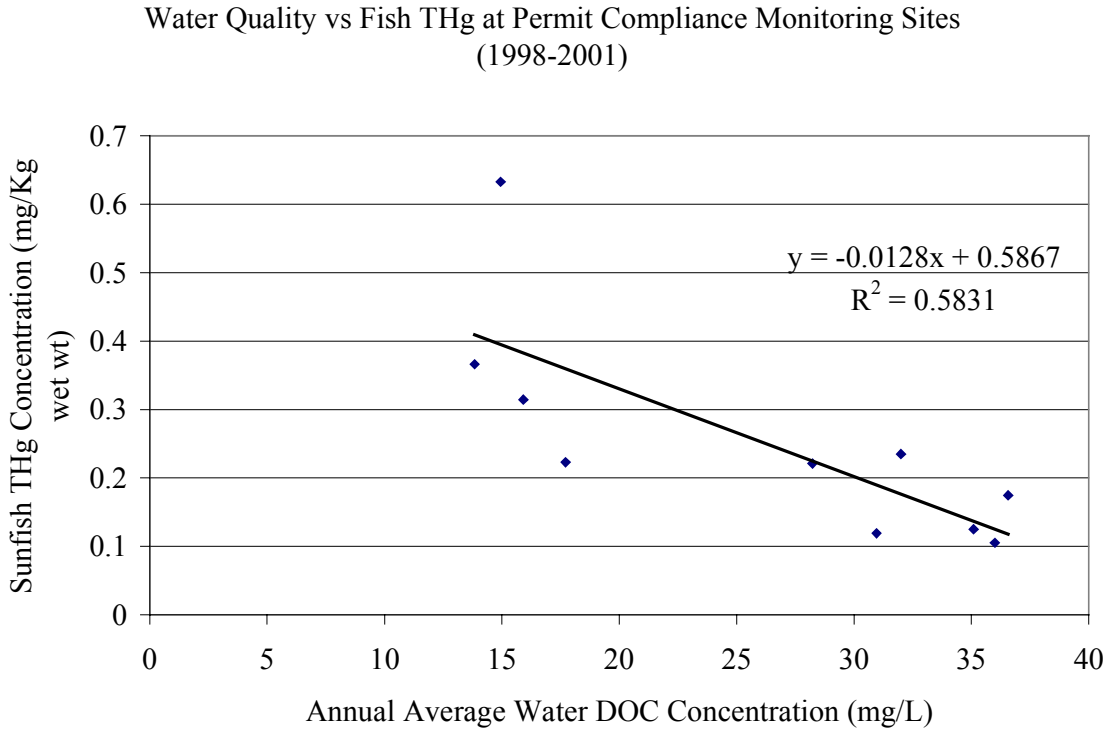

Figure 21. Sunfish THg concentration vs annual average water DOC concentration at permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

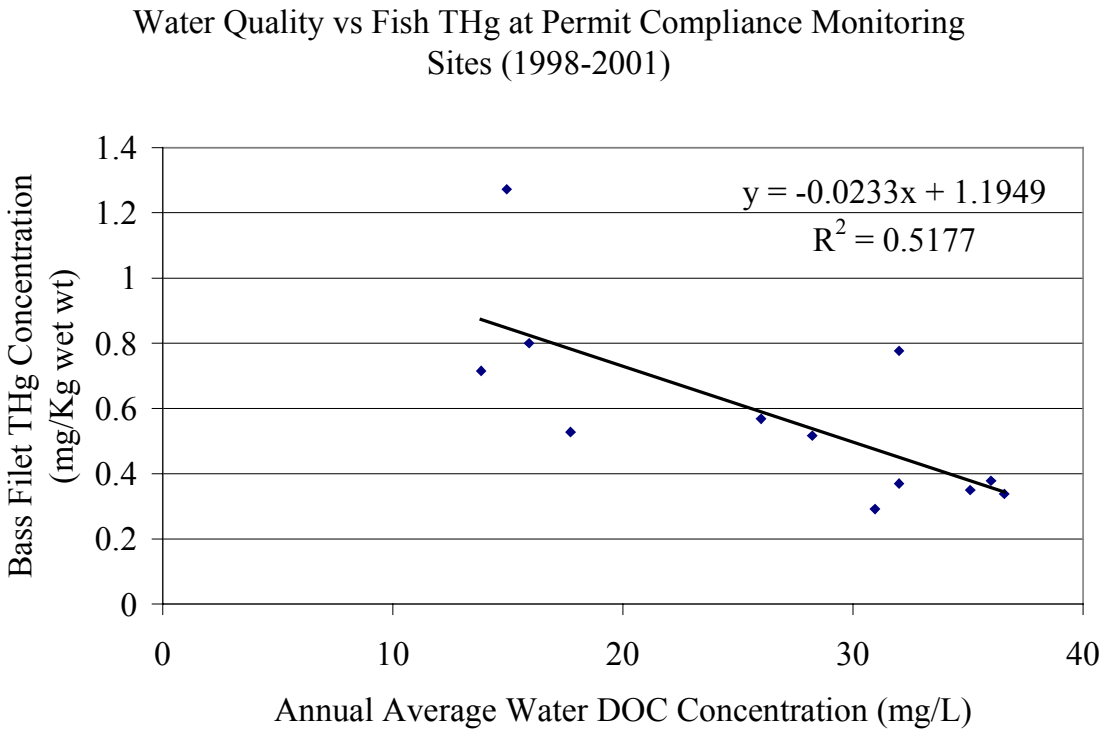

Figure 22. Largemouth bass THg concentration vs annual average water DOC concentration at permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

.

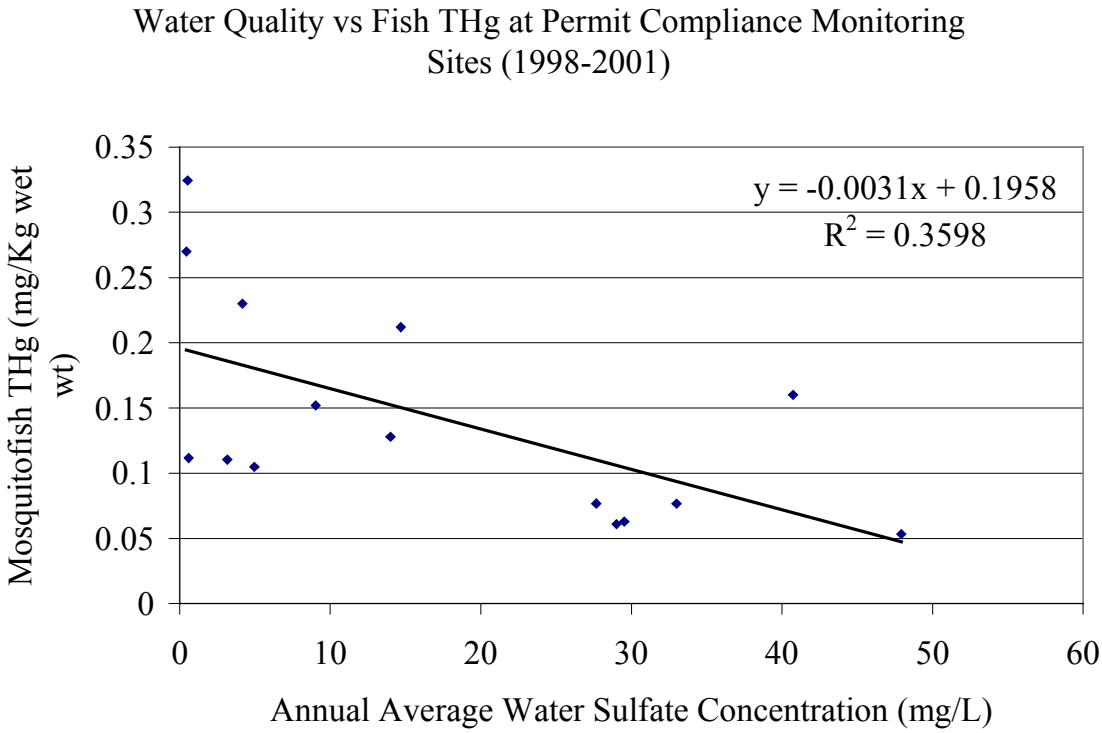

Figure 23. Mosquitofish THg concentration vs annual average water sulfate concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

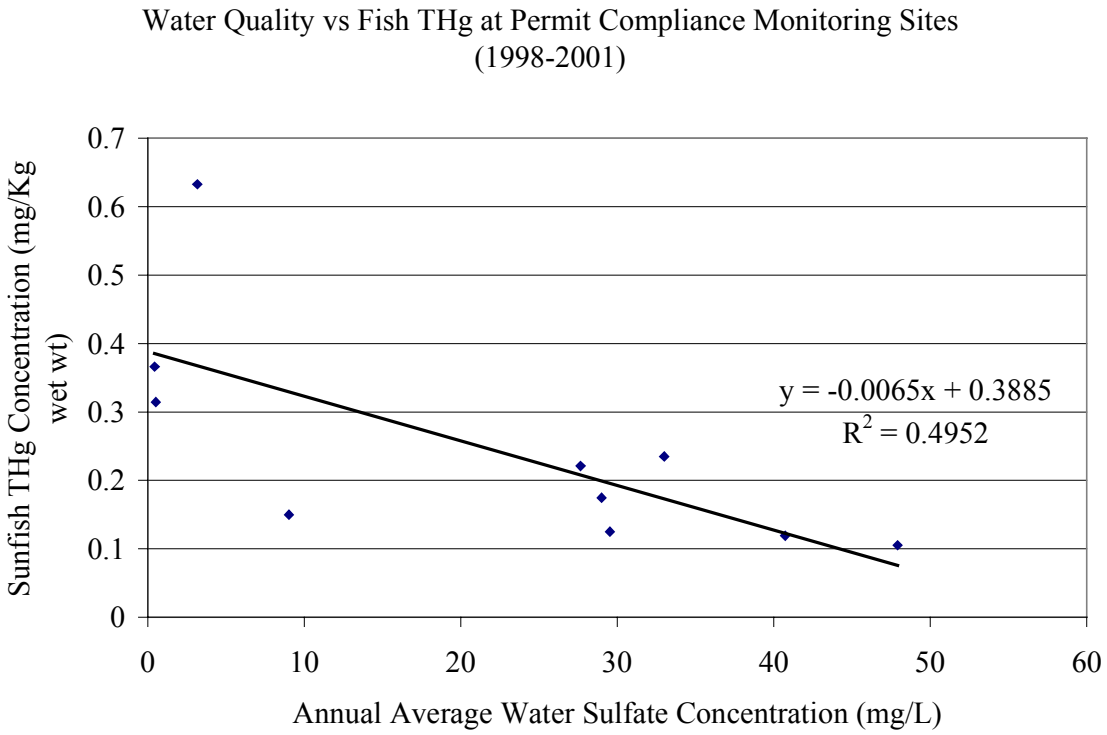

Figure 24. Sunfish THg concentration vs annual average water sulfate concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

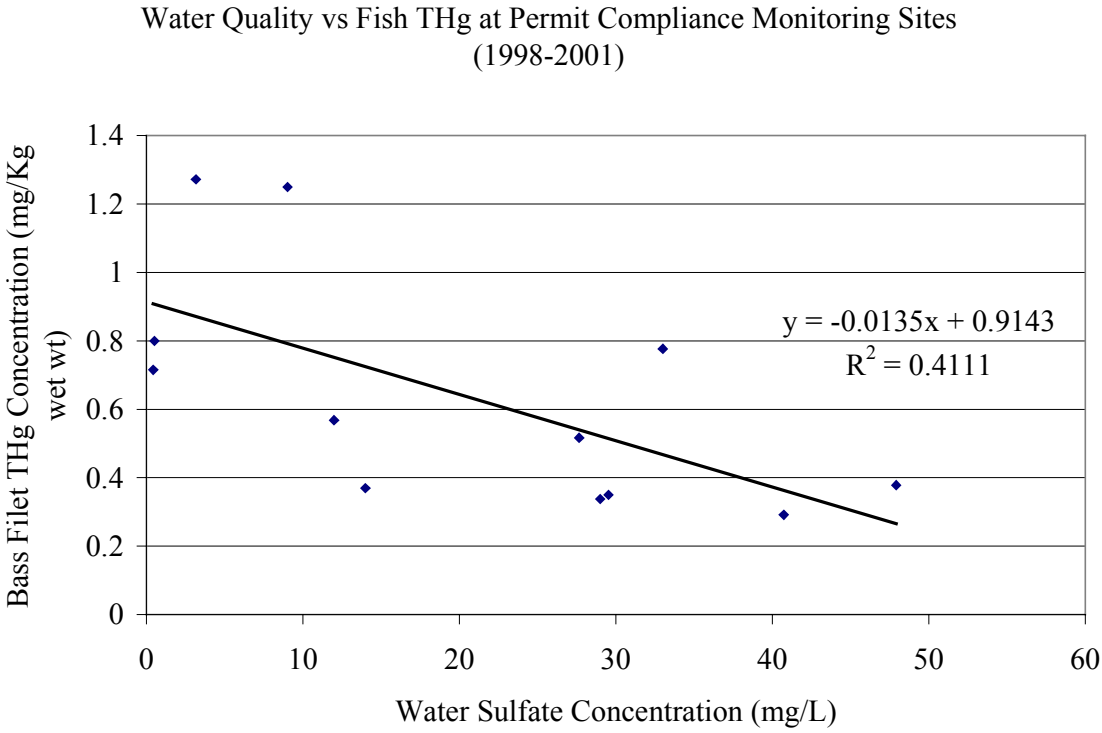

Figure 25. Largemouth bass THg concentration vs annual average water sulfate concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

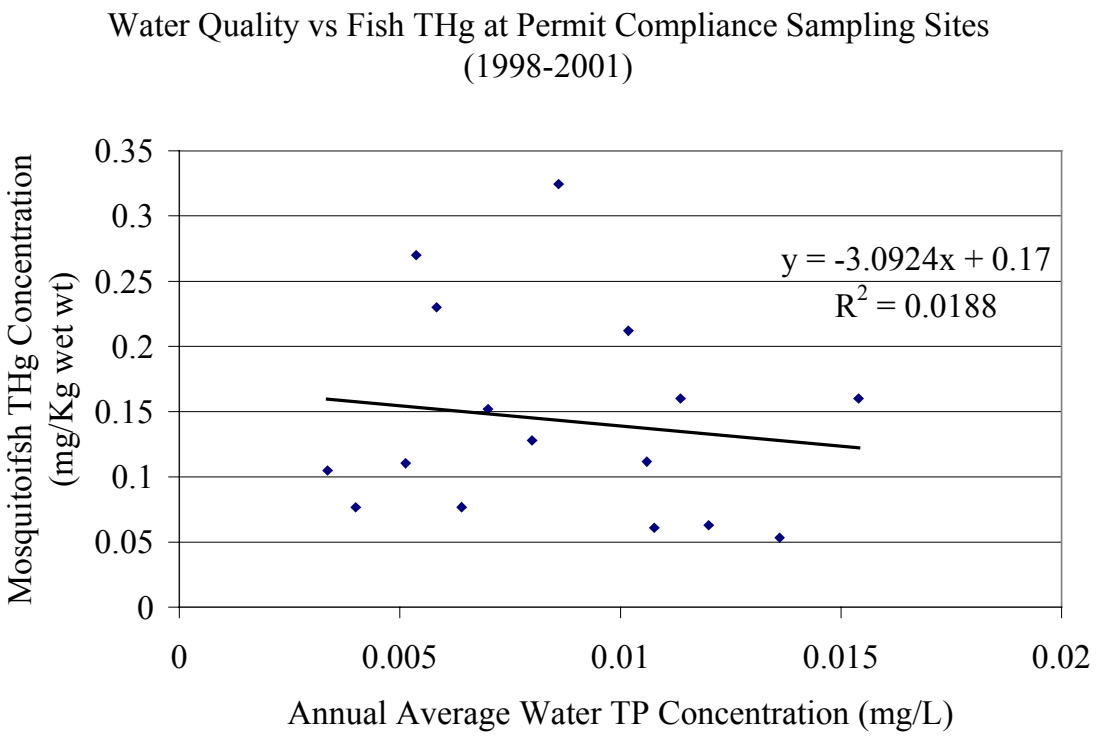

Figure 26. Mosquitofish THg concentration vs annual average water total phosphorus concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

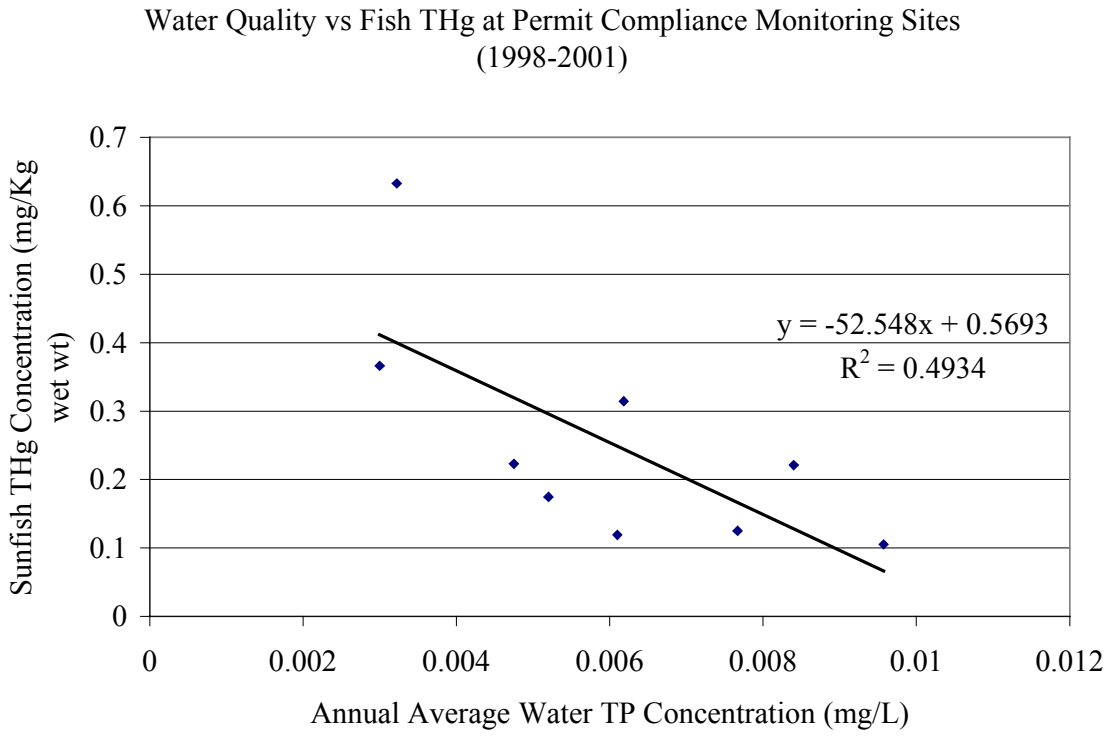

Figure 27. Sunfish THg concentration vs annual average water total phosphorus concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

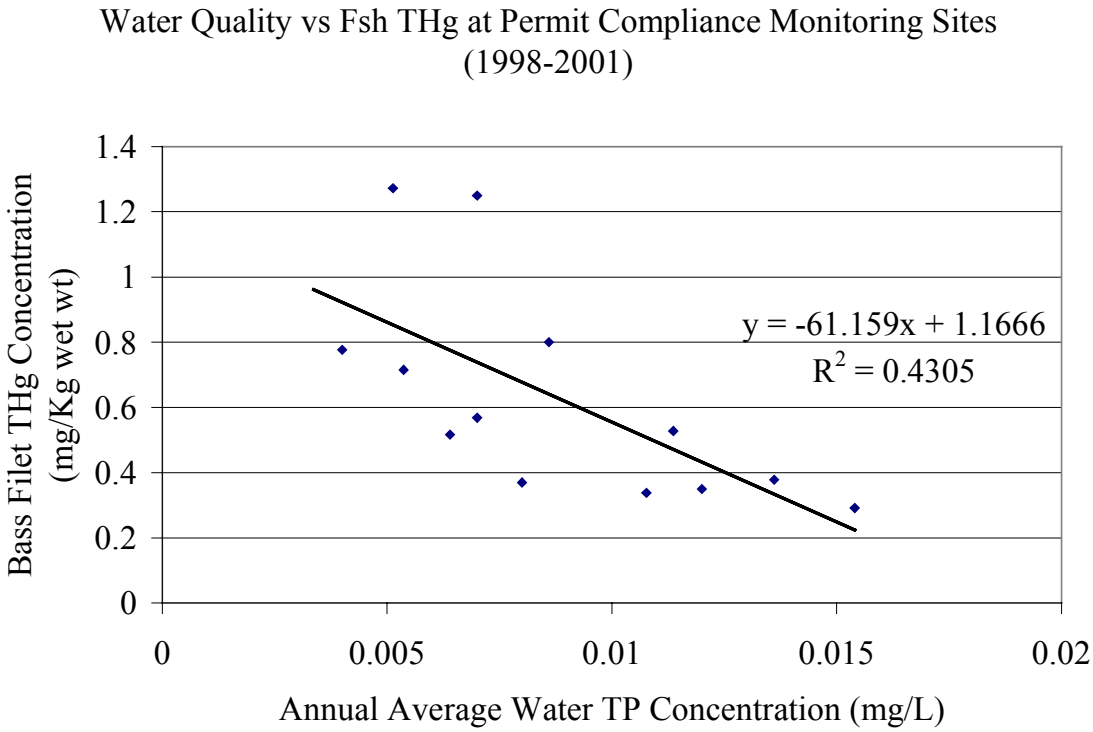

Figure 28. Largemouth bass THg concentration vs annual average water total phosphorus concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

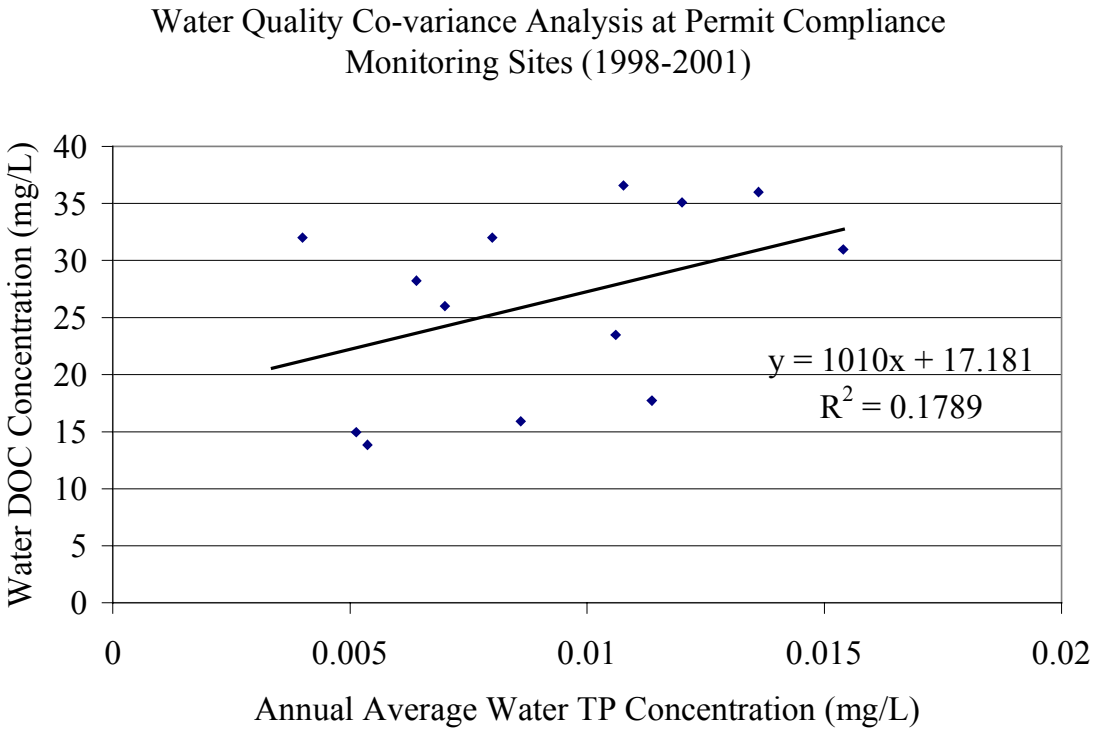

Figure 29. Annual average DOC concentration vs annual average water total phosphorus concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and P3 (ENP)

LABORATORY STUDIES OF THE EFFECT OF P ADDITION ON MEHG BIOACCUMULATION

Miles et al. (2001) measured the adsorption isotherms and calculated Freundlich partition coefficients in the linear concentration range well-characterized laboratory cultures of two green algae species, *Selenastrum capricornutum* and *Cosmarium botrytis*, one diatom, *Thalassiosira weissflogii*, and a blue-green algae, *Schozthrix calcicola*. The log K values reported were:

Table 10. Summary of findings of Miles et al. (2001)

Method	cells/growth status	a	log K +/- SD		log VCF +/- SD		n	slope
Freundlich	S. capricornutum, exp.	A	6.66	0.19	6.81	0.2	6	1.05
Freundlich	S. capricornutum, stat.		6.72	0.39	6.91	0.58	4	0.98
Freundlich	S. capricornutum, exp., P-lim, rep 1	C	5.85	0.01	6	0.08	2	0.78
			5.85		6			
Freundlich	S. capricornutum, exp., P-lim, rep 2	C	5.95		6.61		1	0.94
Freundlich	S. capricornutum, exp., P-lim, rep 3	C	6.07		6.72		1	1.11
Freundlich	Cosmarium botrytis, exp.	A,B	6.74	0.25	5.94	0.69	4	0.92
Freundlich	Schizothrix calcicola, exp.	B,C	6.26	0.25	5.6	0.21	4	0.89
Freundlich	Thallasiosira spp., exp.	A,B	6.72	0.21	5.37	0.04	4	1.08
flow-through	S. capricornutum, exp.		6.54	0.16	6.67	0.13	4	

Reproduced from Miles et al. (2001)

In addition, the researchers evaluated the effect of phosphorus stimulation of MeHg uptake by *Selenastrum* and concluded that the Kp value is generally lower when measured in exponential (log) growth phase sustained by high TP concentrations than in P-limited, static growth phase and that high P also causes structural changes in the cell that reduce MeHg uptake. They also corrected for the DOC present in the algal cultures, recognizing that DOC competes with algal cells for MeHg. However, the KP values were not expressed on the basis of the organic matter or organic carbon fraction of the sorbing medium. Moye et al. (2002) used the same algae cultures, apparatus, and study conditions and various techniques to verify that MeHg uptake was not occurring by passive diffusion, including blocking various metabolic pathways using toxic agents to quench uptake. Among other things, the authors concluded that the uptake rate by the blue-green alga, *Schizothrix calcicola*, which predominates in the low P concentration range of the Everglades, takes up MeHg at a rate one-twentieth that of the green algae species tested.

FIELD MESOCOSM STUDIES OF THE EFFECT OF P ADDITION ON MEHG BIOACCUMULATION

THE ENGLISH-WABIGOON MESOCOSM STUDY

To evaluate the efficacy of intentional eutrophication as a tool for managing MeHg bioaccumulation, Rudd and Turner (1983) dosed mesocosms in impoundments of the English-Wabigoon River system with phosphorus and radioactive 203-Hg(II). The dosing rate used resulted in an initial water column concentration of about 15 ng/L before equilibration with the sediment in the mesocosms. This concentration range should have avoided any artifacts that could be introduced when working with inappropriately high water column concentration ranges, as was often necessary without the use of a radioactive isotope of Hg(II). The authors concluded that, all other factors being equal and not limiting, an increase in algal nutrients was more likely to increase microbial mercury methylation rates and result in stable or increased mercury concentrations in fish, while lowering primary productivity would reduce MeHg production and bioaccumulation at the expense of fish growth rates.

Quoting directly from Rudd and Turner (1983): "The overall effects of increasing primary productivity on Hg concentration of fish appear to be a complex interrelationship between stimulation of the growth rates of fish and microbial Hg methylation rate and, in some cases a change in pH, which may reduce either bioaccumulation efficiency of CH3Hg+ by fish or change the form of methylated mercury produced by microorganisms. Increases in primary productivity that were not large enough to affect ecosystem pH produced the largest increases in Hg concentration of pearl dace and crayfish. These conditions appear optimal for Hg methylation."

MESOCOSM DOSING STUDY OF P VS MEHG PRODUCTION AND BIOACCUMULATION: ACME II

Quoting from Appendix 2B-2 of this Chapter*: "Effect of phosphate enrichment on MeHg production (ACME-SFWMD).* Newman, McCormick and co-workers at the SFWMD conducted phosphate-enrichment mesocosms studies at four sites in the Everglades over the last 2-3 years. These experimental systems provided the opportunity to examine the influence of phosphate on MeHg production, separately from other factors (like sulfate) that co-vary with nutrients across the Everglades. Phosphate might influence net MeHg production directly either through effects on the growth of methylating and demethylating bacteria or by affecting the complexation and therefore bioavailability of Hg. However, experiments in which phosphate was added to sediment cores suggested no direct effect of phosphate on net methylation (Gilmour *et al.* 2000). More likely, phosphate may indirectly effect net MeHg production through enhanced plant growth, leading to higher organic carbon supply to sediment microorganisms and possibly changed redox conditions in sediments. The organic matter supply to sediments affects microbial activity in sediments, and would control sulfate-reduction and sulfide production rates at locations where sulfate is not limiting. Further, dissolved organic carbon acts as a strong ligand for Hg (Ravichandran *et al.* 1998; Benoit et al. 2000) and for MeHg (Hintelmann et al. 1995; Miller et al. 2001) and may inhibit the uptake of MeHg into biota. Nutrient effects on Hg cycling that are mediated through plant growth need to be examined over the longer term. During 2000, ACME scientists worked with Newman and others to measure MeHg concentrations in surface sediments

in the mesocosms. At the time of sampling, the mesocosms were at or near steady state with respect to responses to phosphate additions. This provided the opportunity to examine any effects of enhanced plant growth on net MeHg production.

The SFWMD conducted phosphate-enrichment mesocosms experiments at four sites with a range of in situ phosphate enrichment, from moderately enriched site U3 in WCA 2A, to more pristine sites in central WCA 3A, in central LNWR and Taylor Slough in ENP. While phosphate enrichment changed plant and periphyton communities in the mesocosms significantly, phosphate enrichment changed MeHg concentrations in surface sediments by less than a factor of three at any site. Further, there was no trend across sites in the direction of any MeHg response to PO_4 loading (Gilmour et al., 2001). To put these responses in context, they should be compared with the more than a hundred-fold range in MeHg concentrations and production rates across the Everglades from eutrophic northern WCA 2A to the MeHg maxima in central WCA 3A. These *in situ* mesocosms studies confirm and extend smaller scale studies, showing little direct or indirect effect of phosphate on MeHg production and accumulation in surface sediments."

DARTMOUTH MESOCOSM STUDY

Pickhardt et al. (2002) carried out an outdoor mesocosm experiment to simulate the effect of phosphorus-induced biodilution on MeHg bioaccumulation. In this study, 12 mesocosm tanks were filled with 450 L bedrock well water and leaves from nearby trees were added. Forty-eight hours later phytoplankton innocula were added. Forty-eight hours later baseline nutrients were measured. Twenty-four hours later TP and TN were added in the ratio 30:1 to 12, with six TP doses increasing linearly from 7.4 ug/L to 44.6 ug/L. Nine days later on Day 14 stable isotope-labeled $Hg(II)^{+2}$ and CH_3Hg^{+1} were added to achieve an initial, nominal concentration of 100 ng/L $Hg(II)^{+2}$ and 20 ng/L $CH3Hg^{+1}$. After 24 hours, the concentrations of $Hg(II)^{+2}$ and CH_3Hg^{+1} in each treatment averaged about 1 ng/L and 1.5 ng/L, respectively, with much less variance in the CH_3Hg^{+1} concentration. After 24 hrs, CH_3Hg^{+1} concentrations in algae varied from about 5000 ng/g wet weight at 7.4 ug/L P to about 1,000 ng/g wet wt at 44.6 ug/L P. There was marginal inverse linear dose-response relationship between P concentration and CH_3Hg^{+1} concentration in algal biomass (n = 11, -80.14 (ug P added.liter^{-1}) + 4502, $R^2 = 0.499$, $P < 0.016$) and $Hg(II)^{+2}$ (-917 (ug P added.liter^{-1}) + 45290, $R^2 = 0.623$, $p < 0.004$. Twenty-four hours later, zooplankton were added to the mesocosms. After two weeks, CH_3Hg^{+1} concentrations in Daphnia varied from 5000 ng/g wet weight at 7.4 ug/L P to about 1,000 ng/g wet wt at 44.6 ug/L P. After 3 weeks, $CH3Hg^{+1}$ concentrations in Daphnia varied from 2,500 ng/g wet wt at 7.4 ug/L P to about 1,000 ng/g wet wt at 44.6 ug/L P. There was a marginal inverse linear relationship between TP dose and $CH3Hg^{+1}$ in Daphnia biomass (n=12; -643 (ug P added.liter^{-1}) + 4630, $R^2 = 0.583$, $P < 0.0063$ but not $Hg(II)^{+2}$ (–125.4 (ug P added.liter^{-1}) + 1455, $R^2 = 0.115$ $P > 0.306$). After 3 weeks, –265 (ug P added.liter^{-1}) + 2465, $R^2 = 0.554$, $P < 0.0056$ and $Hg(II)^{+2}$ (-78.7 (ug P added.liter^{-1}) + 1041, $R^2 = 0.213$, $P > 0.130$.

The authors conclude: "Growth biodilution cannot explain our results at 2 weeks, because there were no differences in zooplankton density across treatments even though marked differences in MeHg levels of individuals were evident. Growth dilution did not occur by means of increasing body size either, because there were no significant body-size differences in Daphnia with increasing nutrient addition 2 and 3 weeks after spike additions (see Fig. 2F for lengths at week 3). However, 3 weeks after the zooplankton addition there was a marginally significant trend for lower MeHg concentrations in treatments with higher Daphnia densities (Fig 2E.). This pattern provides some support for the hypothesis that growth biodilution leads to lower mass-specific CH_3Hg^{+} in Daphnia at high density over time."

From the author's summary of the results, it would appear that even under these ideal conditions, the observed biodilution effect was weak. In addition, there are problems associated with extrapolating this "marginally significant trend" to real aquatic ecosystems in general or to the Everglades in particular. The Pickhardt et al. experimental design was intended to simulate deep lakes. No sediment was added to the tanks, there was no significant biological means for MeHg to be produced from inorganic mercury in the tanks as in the Everglades, MeHg production has been measured in periphyton mats, but there were no periphyton or floating aquatic macrophytes in the tanks. In addition, there were differences in water chemistry that could have important effects on the results. For example, DOC is present in sufficiently high concentrations in the Everglades that it competes effectively with organic particles for Hg((II) and MeHg. This weakens the link between plant production and the concentrations of MeHg in water, sediment, and biota. The addition of leaves resulted in uncontrolled production of organic particles and DOC unrelated to algae production. It is possible that there was some uncontrolled, *in situ* MeHg production from the Hg(II) on the leaves or in wet and dry atmospheric deposition, and that it increased in rate with increasing P dose, such that the slope of the correlation between P dose and MeHg with algae decreased from week 2 to week 3. As such, the experimental design may have simulated some of the conditions in deepwater lakes, but it could not have simulated the conditions in the highly organic, shallow wetlands of the Everglades, nor was it designed to do so.

MASS BUDGET ANALYSIS OF BIODILUTION HYPOTHESIS AS APPLIED TO THE WCA-2A NUTRIENT GRADIENT

In an attempt to quantify the degree to which the loss of biodilution with distance down the WCA-2A nutrient gradient was causing or contributing to the observed increase in the average THg concentrations in mosquitofish with distance, the District undertook a mass budget analysis of mercury storage and turnover using measured values of coverage, standing crop, and production for cattail, sawgrass, and periphyton at the most eutrophic site, F1, and the most oligotrophic site, U3, along the nutrient gradient where USEPA 4 had collected its water quality and mosquitofish data in 1993-1994.

If "classical" biodilution were occurring along the WCA-2A nutrient gradient, then the concentrations of THg and MeHg would be lower in the water column, sediment, plants and fish at F1, the most nutrient-enriched or eutrophic site, and highest in the water column, sediment, plants and fish at U3, the unimpacted reference site where nutrient-poor or oligotrophic conditions prevail. Certainly water, sediment and fish concentrations all increase with downstream distance along the nutrient gradient, suggesting a strong biodilution effect. However, the plant data are not so compelling.

LIGHT-LIMITATION EFFECT

The study sites along the WCA-2A nutrient gradient are depicted in **Figure 30.** A calculation of the magnitude of biodilution for THg was carried out for macrophytes (cattail and sawgrass) and periphyton species at four sites along the nutrient gradient: F1, E1, U3 and U1. The spatially weighted-average plant production was calculated by multiplying the spatial coverage of each plant by the corresponding plant density and the plant production or turnover rate (McCormick et al., 1998; Miao and Sklar, 1998). The THg concentration in each plant type (D. Krabbenhoft, USGS, unpublished data, 1999) was then multiplied by its appropriate spatially-weighted average turnover rate to obtain the THg flux through each plant type. The results of the macrophyte and periphyton storage and turnover calculations are displayed in **Table 10**.

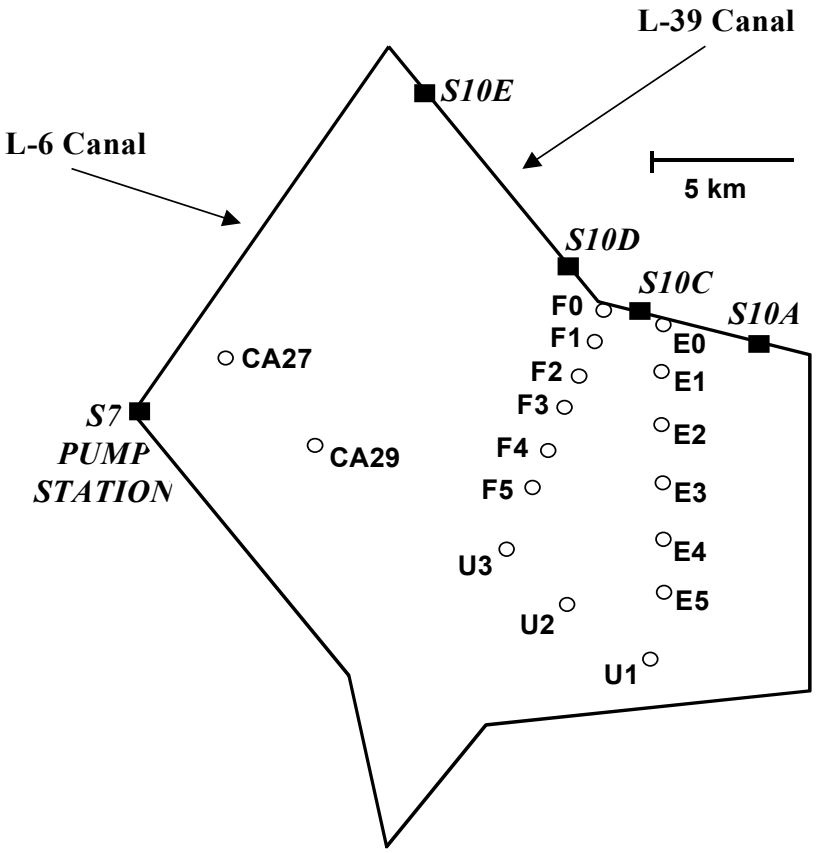

Figure 30. "E" and "F" Transect Research Sites along a well-studied nutrient gradient in Water Conservation Area-2A in the northern Everglades.

As expected, macrophyte density, production and turnover are higher at the eutrophic sites, as is the THg cycled through macrophyte biomass. Macrophyte cycling of THg through macrophyte biomass decreased by 35 percent while the THg concentration more than tripled, so the macrophytes are behaving at least qualitatively as would be predicted by the biodilution hypothesis. However, due to the low concentrations of THg in macrophyte biomass, the quantity

of THg being cycled through plant biomass (7-10 ug/m^2-yr) is small compared with the estimates of the combined wet and dry deposition flux to the Everglades (35 to 45 ug/m^2-yr). The corresponding average flux of THg from the sediment has been observed to be negative, that is, the overlying water is usually saturated with THg relative to the pore water in peat soil (G. Gill and co-workers as discussed in Gilmour et al., 1998b). So the throughput and cycling of THg through plant biomass is probably being driven primarily by atmospheric deposition.

Table 11. Primary producer biomass, THg concentrations and flux rates under a high and low nutrient regime

		Coverage-Weighted Biomass (g dry/m^2	THg (ng/g dry)	THg Storage (ng/m2)	Plant Biomass Turnovers Per Year (g dry/ g dry-yr)	THg Cycled Through Plant Biomass (ug/m^2-yr)
Eutrophic Sites						
Macrophytes		920	2.1	1900	5.0	9.5
Periphyton		1.4	205	280	150	42
Oligotrophic Sites						
Macrophytes		520	6.7	3500	2.0	7
Periphyton		370	39	14,400	9.1	130

In addition, some, perhaps substantial fraction of the THg in macrophyte leaves and stems could originate with the soil and be recycled directly back to the soil without ever participating in any of the other mercury biogeochemical processes leading to MeHg production.

Perhaps surprisingly, the coverage-weighted periphyton biomass was more than two hundred times higher at the oligotrophic sites than the eutrophic sites, resulting in a mercury flux through periphyton biomass at the oligotrophic sites three times that at the eutrophic sites. This is the opposite of the relationship expected for biodilution mediated by water column phosphorus. Consistent with the greater turnover of periphyton at the oligotrophic sites, the THg concentrations at the oligotrophic sites were one-fifth those at the eutrophic sites. Despite the lower THg concentration, the oligotrophic periphyton stored nearly 40 times more THg in standing crop biomass per unit area than the eutrophic site. In addition, the turnover of THg through periphyton biomass at the eutrophic site is about equal to the annual average wet and dry deposition flux of Hg(II) to the Everglades, while that at the oligotrophic site is about four times that value. This strongly suggests that periphyton is only a temporary storage depot for Hg(II) and MeHg and that a substantial portion of the THg sorbed to periphyton biomass is returned to the water column during biomass decomposition; otherwise, there would be no way to sustain the calculated THg turnover rate at U3 without an external deposition flux of THg that would be inconsistent with the peat accretion profile (Delfino et al., 1993) and mercury deposition profile (Vaithiyanathan et al., 1996).

Based on the preceding analysis, it is clear that the oligotrophic site has a higher biodilution factor than the eutrophic site, contrary to the phosphorus-mediated, classical biodilution hypothesis. This counterintuitive result probably arises from the suppression of periphyton production through light limitation (Grimshaw et al., 1997). Light limitation occurs at the highly

eutrophic sites because the dense canopy of living and dead emergent macrophyte biomass shades the water column. Grimshaw et al. (1997) found a significant decrease in net primary production of periphyton under *Typha* stands when compared to open waters and *Cladium* stands. As a result of light limitation, the "classical" link between eutrophication and biodilution is broken and is no longer directly applicable to the more eutrophic sites along the WCA-2A nutrient gradient. The net result of this light limitation effect is that the greater standing crop density and higher turnover rate of plant biomass is occurring at the oligotrophic site (McCormick et al., 1998), and this is apparently resulting in lower concentrations in plant biomass than at F1, despite the fact that the concentration of THg in the surrounding water is higher on average at U3 than at F1.

The expected relationship between biodilution and eutrophication appears to be present in the macrophyte community. Macrophyte biomass is greater and the calculated THg turnover rate is higher at the eutrophic site than at the oligotrophic site. In addition, the concentration of THg appears to increase in peat soil as water column phosphorus decreases (Delfino et al., 1993; Vaithiyanathan et al., 1996), suggesting that the rate of peat formation and dilution of the rainfall mercury flux increases as water column phosphorus increases. However, the actual difference in the THg concentration in surficial soils (0 - 5 cm) at F1 and U3 is small (108 vs 130 ug/Kg wet weight on a bulk density-weighted average basis, calculated from data supplied by Gilmour et al., 1999) and some of the apparent effect of biodilution in the deeper sediments at F1 may be a consequence of the more efficient mining of Hg(II) and MeHg by cattail roots than sawgrass roots and the three-fold higher Hg(0) evasion rate from cattail leaves than sawgrass leaves (Lindberg et al., 1999). It is also possible that the slight decrease in the average DOC concentration between F1 (45 mg/L) and U3 (38 mg//L) results in a greater proportion of the Hg(II) sorbing to settling organic particles, with a concomitant increase in the average concentration of Hg(II) and MeHg on those particles. This effect may be enhanced by the slow shift from the more aromatic DOC in EAA runoff, with a higher affinity for Hg(II) and MeHg, to a more aliphatic DOC produced internally, with a lower affinity for Hg(II) and MeHg (G. Aiken, USGS, personal communication, 2002). If biodilution is the cause of this difference, this is a weaker manifestation of the biodilution effect. Due to the apparent weak relationship between soil THg and MeHg production, this manifestation of the biodilution effect probably has only a second-order effect on MeHg bioaccumulation in Everglades fish. Nevertheless, this manifestation of the biodilution effect is taken into account by the modified E-MCM(II) (Tetra Tech, Inc., 2002).

CATTAIL BIOCONCENTRATION EFFECT

During a study of the effect of dryout and burn on the Everglades mercury cycle in July of 1999, the USGS-Madison collected samples of surface water, pore water, sediment, plants, and mosquitofish at ten interior marsh sites for THg and MeHg analysis. From north to south, those sites were ENR 103, WCA-2A-F1, LOX, WCA-2A-U3, WCA-2BS, WCA-3A-33, WCA-3A-15, WCA-3A-TH, WCA-3A-TS7, and WCA-3A-TS9. Although this was a one-time "snapshot" of the conditions at these sites, the concentrations of THg and MeHg in the tissues of rooted plants most likely represented an integration of the long-term average uptake of Hg(II) and MeHg from soil over the period of time required to produce the leaves, stems, and roots of the plant sampled in the study and not a short-term response to post-dryout/burn conditions (Krabbenhoft et al., 2000).

Some submergent rooted macrophytes have been shown to take up Hg(II) and MeHg primarily from the water column (Ribeyre and Boudou, 1994). It is not known *a priori* whether cattail or

sawgrass obtain the Hg(II) and MeHg in their tissues primarily from the water column or from the sediments. If the former, then the high densities and turnover rates of cattail at highly phosphorus-enriched, eutrophic sites such as F1 could, in theory, result in a substantial biodilution of the Hg(II) and MeHg in the water column. However, as noted above, on a mass balance basis, only about one-third of the Hg(II) in atmospheric deposition flux is cycling through cattails at F1, so, in practice, even if cattail were absorbing Hg(II) and MeHg from the water column, it would have a minimal effect on the mercury throughput for site F1. Evidence to support the likelihood that the plant tissue Hg(II) and MeHg are being obtained primarily from the sediment comes from the study of enhanced evasion of sediment Hg(0) from dense cattail and sawgrass stands relative to open water (Lindberg et al., 1999; Lindberg et al., 2002, in press).

To evaluate the efficiency of uptake of rooted plants from soil, the absolute concentrations of THg and MeHg, the %MeHg and %Hg(II) were divided by the corresponding concentrations or percentages in the top 10 cm of underlying soil based on data collected from March 1995 through January 1999 by the U.S. Geological Survey in Middleton, WI, as part of the Aquatic Cycling of Mercury in the Everglades (ACME) Study. The absolute concentrations of MeHg in tissues, %MeHg in tissues, and the ratio of the %MeHg in tissue-to sediment are displayed in Figures 31, 32, and 33 for sawgrass and 34, 35, and 36 for cattail. The tendency for both Hg(II) and MeHg to preferentially concentrate in the tissues in the order of green leaves < senescent leaves < fibrous roots < tap roots suggests that the sediment is the primary source of both Hg(II) and MeHg in cattail and sawgrass tissues. Interestingly, the ratio of %MeHg in cattail tissues relative to sediment appears to increase with increasing total phosphorus in water and sediment from WCA-3A-33, which is in the northern portion of WCA-3A, to F1, which is 1.8 km south of the S-10 C structure on the L-39 levee in the northern portion of WCA-2A, to ENR 103, which is in the northern portion of one of the upper treatment cells in the Everglades Nutrient Removal (ENR) Project.

Unfortunately, cattail and sawgrass were collected simultaneously only at 3A-33, which precludes a robust comparison of their relative capacities for bioconcentration or biodilution of Hg(II) and MeHg from the sediments under a wide variety of conditions. A comparison of the ratios of THg, MeHg, %MeHg, THg sediment bioconcentration factor (SBCF) and MeHg SBCF in cattail relative to sawgrass at 3A-33, where cattail was found to be about twice as efficient as sawgrass in taking up MeHg from the soil, based on the ratio of %MeHg in plant tissue to % MeHg in the sediment in which the roots are growing. This is less than the ratio of their transpiration rates, which is about 3-to-1 (Koch and Rawlik, 1993).

The question then arises whether the more rapidly growing cattail collected at one of the most eutrophic sites, WCA-2A-F1, are biodiluting or bioconcentrating the Hg(II) and MeHg from the soil relative to the more slowly growing sawgrass collected at the highly oligotrophic site, WCA-2A-U3. To answer this question, the ratios of cattail to sawgrass for the above indicators of biodilution or bioconcentration were again evaluated. While the ratio of the %MeHg in sawgrass leaves to the average %MeHg in the top 10 cm of soil at U3 is about 2.6-to-1, that same ratio in F1 cattail is 26-to-1. This despite the fact that the concentration of MeHg in surficial soils at U3 are about three to four times the concentrations at F1 (Krabbenhoft et al., 2000; Gilmour et al., 1999). While both sawgrass and cattail appear to be bioconcentrating MeHg in their green leaves relative to the sediment in which they grow, F1 cattail are about 10 times more efficient than U3 sawgrass. For senescent leaves the ratio of the %MeHg ratios decreases to about 7-to-1, suggesting that MeHg is mobilized preferentially from the senescing leaves relative to Hg(II) during the resorption process. This is still a substantial discrepancy between the dominant rooted plant species at the two sites. Coupled with the much faster growth rate of F1 cattail, the loss of MeHg mined from the sediment during leaf decomposition at F1 could make a substantial

contribution to the flux of MeHg to the water column or directly into the detrital food chain. However, it is also possible that the MeHg in decomposing senescent leaves is sufficiently refractory that it does not make a substantial contribution to the flux of MeHg to the water column or the detrital food chain. At present, there are insufficient data to answer this question with the required accuracy and confidence level.

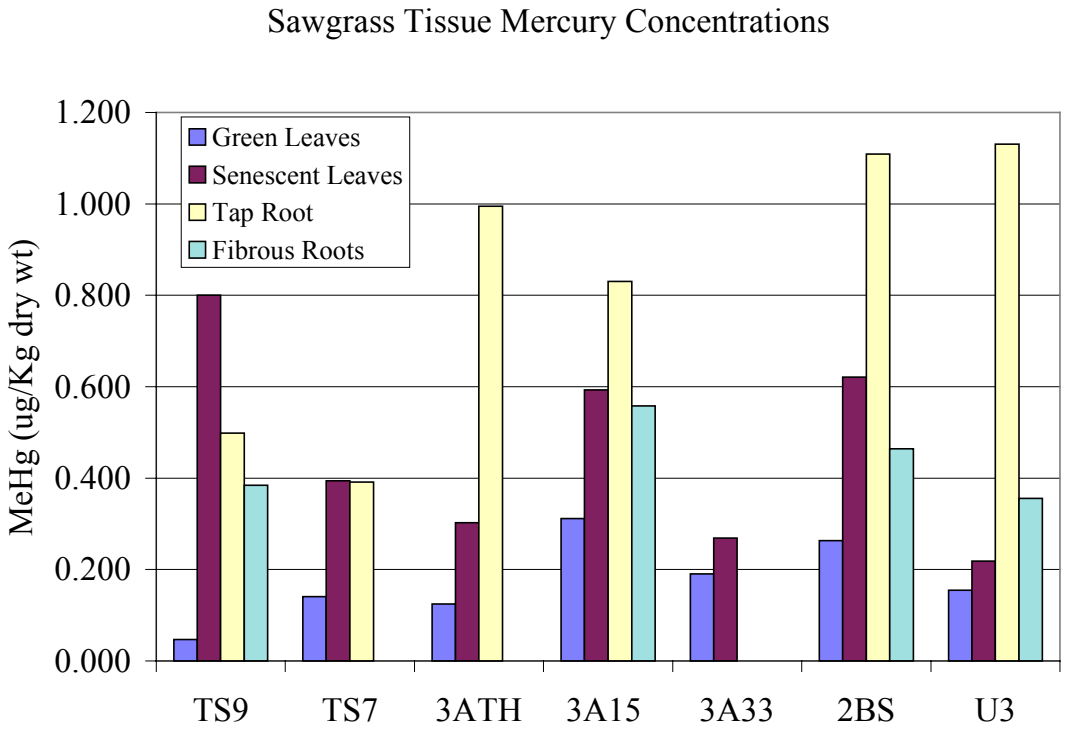

Figure 31. MeHg concentration (ug/Kg dry wt) in sawgrass tissue collected from various sites in the Everglades following a severe dryout and burn event in July 1999.

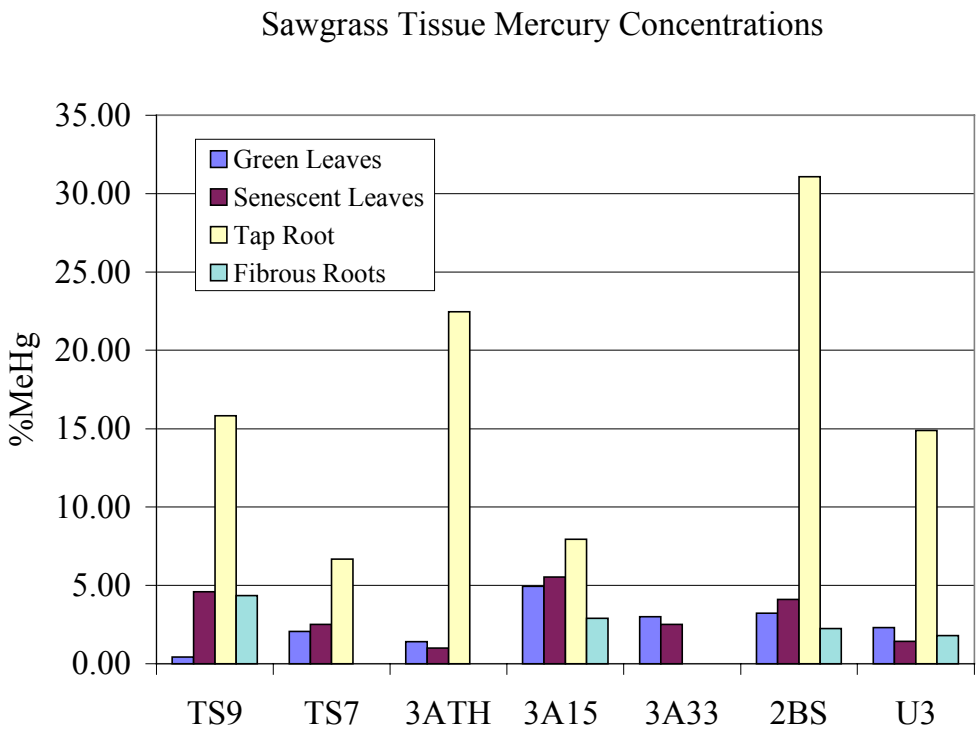

Figure 32. %MeHg in sawgrass tissue collected from various sites in the Everglades following a severe dryout and burn event in July 1999.

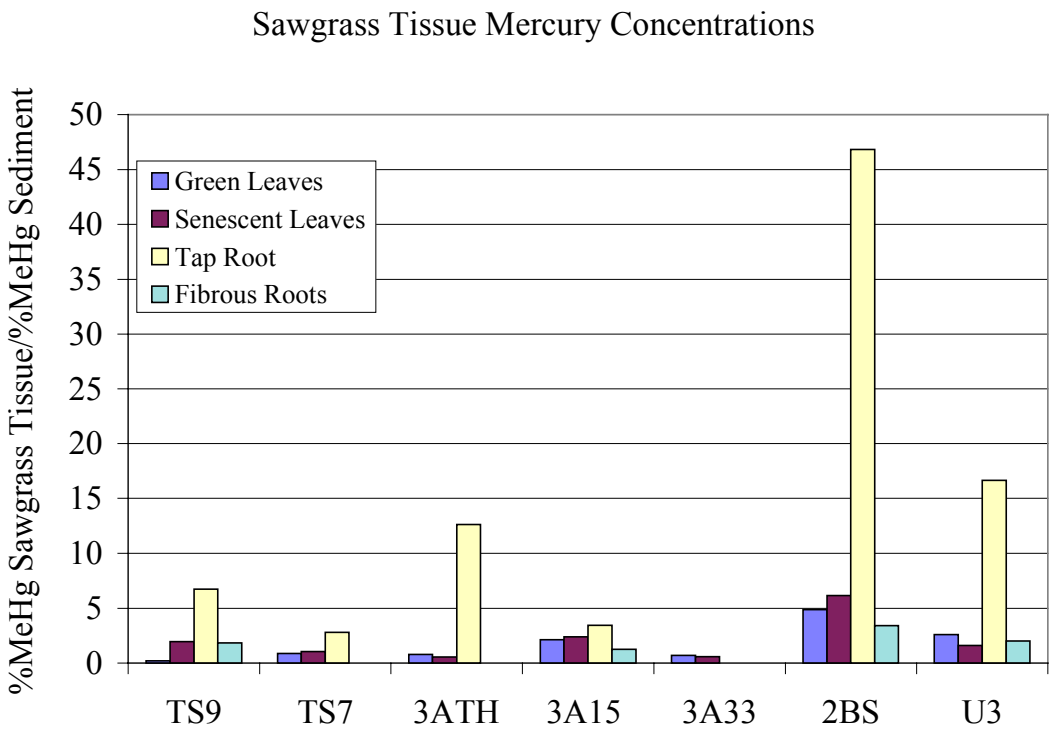

Figure 33. Ratio of %MeHg in sawgrass tissue collected from various sites in the Everglades following a severe dryout and burn event in July 1999 to average %MeHg in sediments from same sites for period 1995-1999.

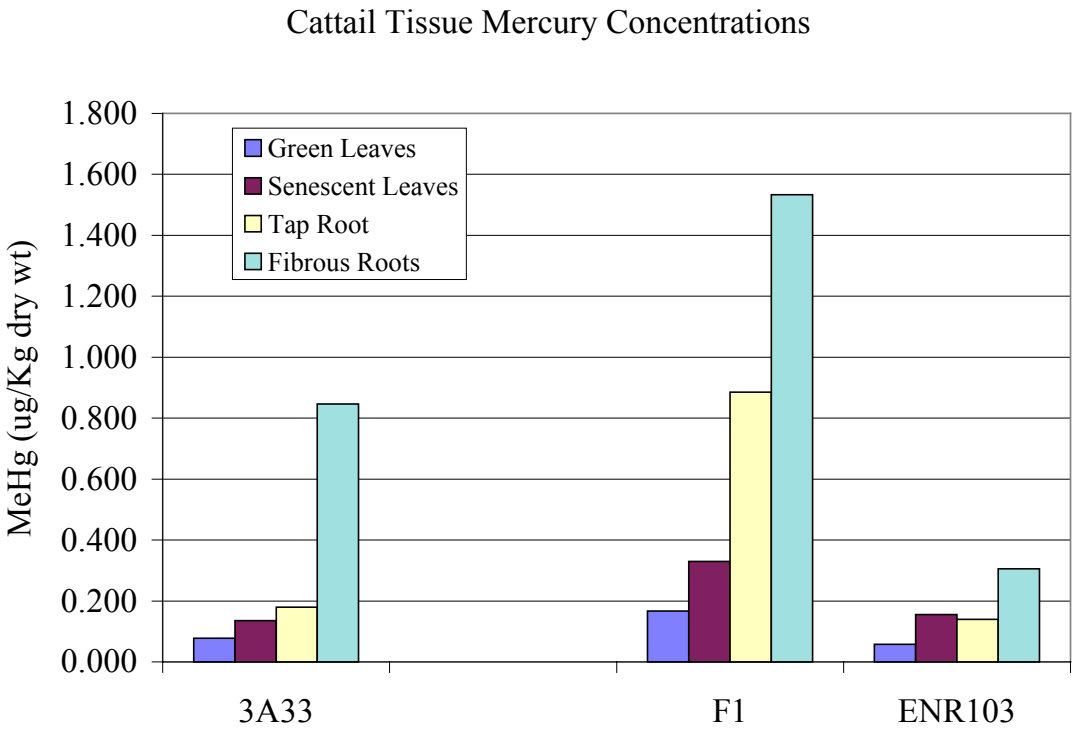

Figure 34. MeHg in cattail tissue (ug/Kg dry wt) collected from various sites in the Everglades following a severe dryout and burn event in July 1999.

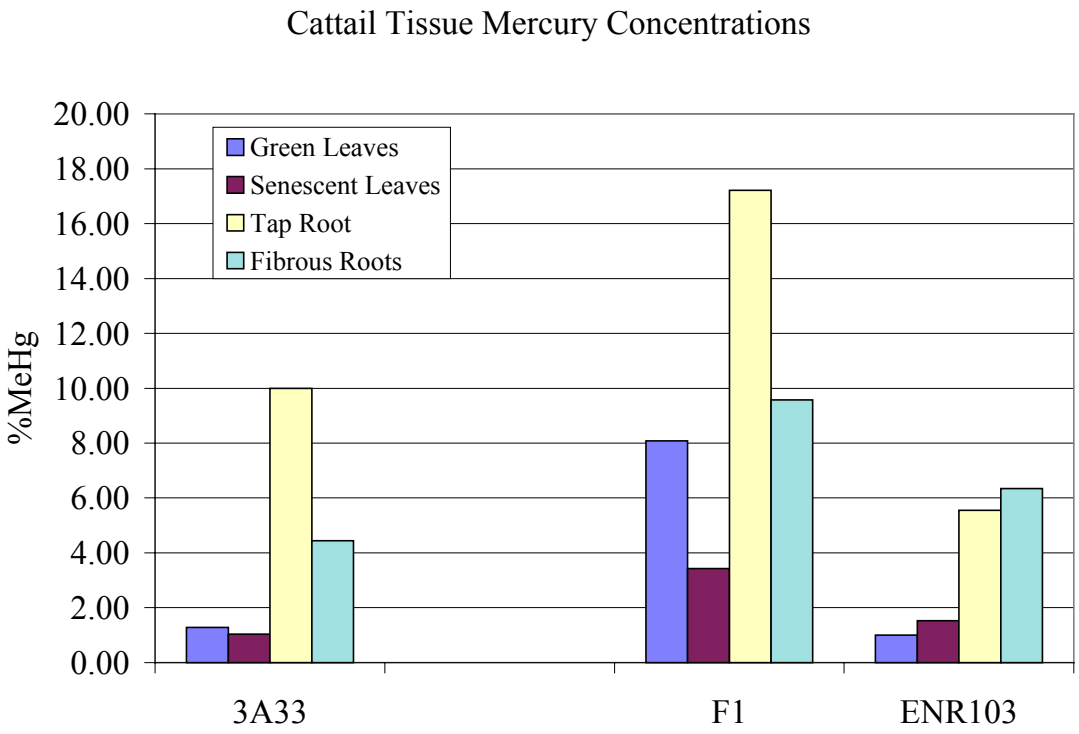

Figure 35. %MeHg in cattail tissue (ug/Kg dry wt) collected from various sites in the Everglades following a severe dryout and burn event in July 1999.

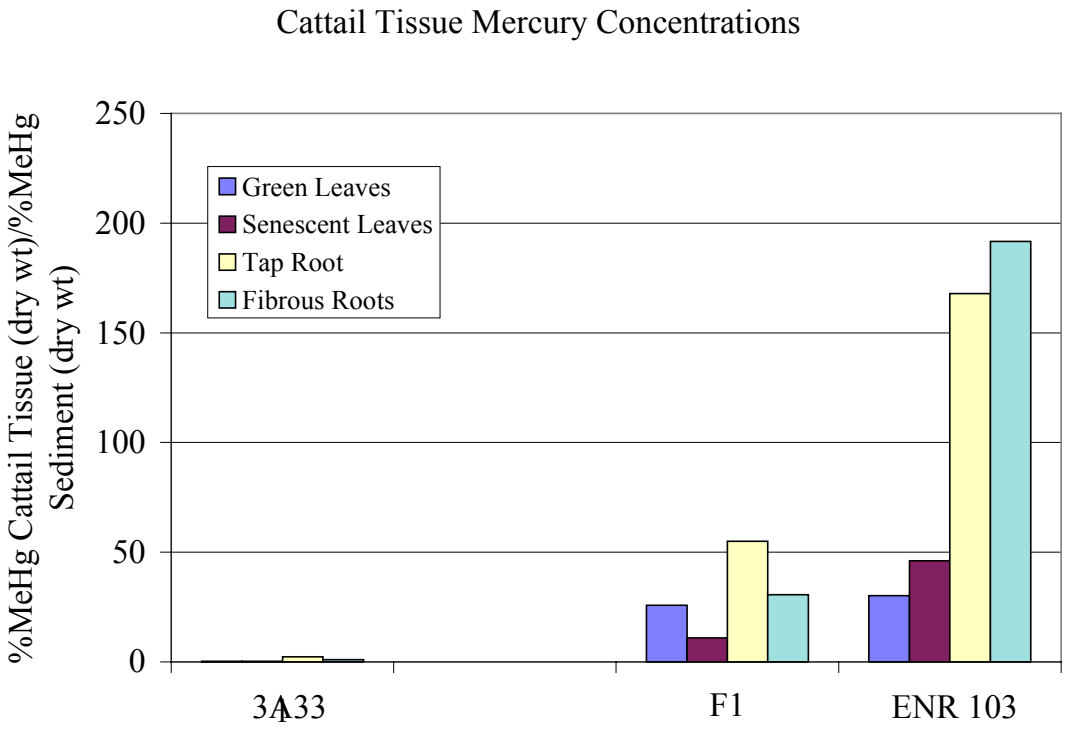

Figure 36. Ratio of %MeHg in cattail tissue collected from various sites in the Everglades following a severe dryout and burn event in July 1999 to average %MeHg in sediments from same sites for period 1995-1999.

Based on the light limitation effect occurring along the WCA-2A nutrient gradient, **classical biodilution cannot be the explanation for the observed increase of MeHg bioaccumulation in mosquitofish with downstream distance along the WCA-2A nutrient gradient.** The apparent inverse relationship between water column total phosphorus and MeHg bioaccumulation in mosquitofish is most likely correlation, not causation. Moreover, the ability of cattail to bioconcentrate rather than biodilute MeHg from sediment is likely to increase the flux of MeHg into the most eutrophic sites where cattail stands are densest and have the highest productivity, rather than decrease the concentrations of MeHg in water, sediment, and biota due to a biodilution effect.

Based on everything else that has been learned about the influence of surface water, pore water, and sediment solids chemistries on MeHg production, decomposition, transfer to benthic organisms, and bioaccumulation up the detrital food chain, it is much more likely that the nearly four-fold increase in the sediment MeHg concentration between F1 and U3 (Gilmour et al., 1999) translates into a nearly four-fold increase in the concentration of MeHg in the benthic organisms living on or in the sediments. It can be conjectured that this increase is then further magnified by the addition of about one step in the food chain between mosquitofish and the sediment.

Evidence for this food chain conjecture is two-fold. First, the guts in mosquitofish from F1 tend to contain a much higher proportion of sediment and benthic invertebrates, while the guts of mosquitofish collected at U3 tend to contain a greater proportion of periphyton and invertebrates living on the periphyton mats (Cleckner et al., 1998). While it is unlikely that the mosquitofish are digesting the periphyton, it is likely that they derive some sustenance from the digestion of the bacteria that are decomposing the dead periphyton tissue. The other line of evidence comes from the observation that the ratio of MeHg/THg in mosquitofish increases from < 50% at F1 to > 85% at U3 (P. Rawlik, SFWMD, personal communication based on unpublished District data). However, the inferred increase in the length of the mosquitofish food chain between F1 and U3 has not yet been detected using carbon and nitrogen isotope shift data from mosquitofish collected by USEPA 4 during its fall and spring campaigns in 1994-1996 (C. Kendall, USGS, personal communication, 2001). The analysis of mosquitofish collected quarterly by the District at F1 through F5 and U3 from November 1998 to August 2000 may permit a reduction in the variability in the data attributable to seasonal trophic dynamics related to water depth-duration and water temperature and unrelated to the degree of eutrophication *per se*.

MECHANISTIC MODELING ANALYSIS OF THE BIODILUTION PHENOMENON

Through a contract with the USEPA, Tetra Tech previously adapted an existing dynamic model of mercury cycling in lakes (D-MCM) (Tetra Tech 1999a) to apply to conditions in Everglades marshes, resulting in the Everglades Mercury Cycling Model (E-MCM) (Tetra Tech 1999b). Original model development was carried out using Water Conservation Area 3A-15 as the first calibration site. E-MCM was also applied to WCA 3A-15 to predict the response of fish mercury concentrations to changes in atmospheric Hg deposition, as part of a pilot mercury TMDL study for the USEPA (Tetra Tech 2001).

D-MCM is a fully dynamic, stirred tank model with exchange between a stratified sediment and a well-mixed water column. E-MCM (I) was developed by USEPA's Office of Research and Development (Ambrose and Araujo, 1998) to support management decision-making regarding the effects of the Everglades Construction Project (ECP) and the Comprehensive Everglades Restoration Plan (CERP) on Hg(II) accumulation and MeHg bioaccumulation in constructed wetlands and/or the downstream environment. During Phase I, modifications to E-MCM (I) were made so that it could operate in a probabilistic (Monte Carlo) as well as deterministic mode. The stirred reactor model was applied to the Everglades Nutrient Removal (ENR) Project, where extensive mercury mass budget studies had been carried out. In Phase II, E-MCM (I) was modified to make it possible to simulate a flow path through the Everglades (cells-in-series mode). Modifications to format the EMCM (I) input file to accept output directly from the District's ELM had to be postponed, however. The cells-in-series version was then applied to the problem of simulating the effect of changes in Hg(II) loading, flow, and water quality associated with the ECP on MeHg production and bioaccumulation in a nutrient-impacted area in Water Conservation Area 2A (WCA-2A). During Phase III, a mass balance model describing a simplified sulfur cycle in the Everglades was developed for inclusion in E-MCM. The objective of this simplified model was to give E-MCM the ability to simulate sulfur mass balance dynamics including fundamental sulfur biogeochemical processes. In addition, E-MCM was modified to accommodate a bottom-up approach to simulating bioenergetics and interactions across trophic levels in the cycling and transfer of mercury through aquatic biota.

Finally, additional management scenarios were simulated in Phase III for the trophic gradient across Water Conservation Area 2A. These scenarios differed from the initial set of management scenarios used in Phases I and II, in that simple assumed relationships between total phosphorus concentrations in surface waters and system productivity and particle budgets were developed and embedded in the model simulations. Furthermore, the initial management scenarios had assumed methylation rates supplied for each scenario by the SFWMD. In the Phase III scenarios, a relationship for methylation constants as a function of total phosphorus concentration in surface waters was developed on the basis of methylation rates that had been calibrated to sites with different phosphorus levels.

Phase III scenarios evaluated the effect of reducing surface water TP from 175-180 ppb at the most eutrophic, well-studied site, F1, in 1994-1997 to 70 ppb in 2002 and 10 ppb in 2006. Depending on the calculated peat accretion rate, the results of this analysis indicated that there would be virtually no effect of P reduction on MeHg bioaccumulation or, at most, a one and one-half to two and one-half times increase in MeHg in top-predator fish with the existing flow and a two-and one-half to three-and one-half times increase if the flow is halved, as is expected with the diversion of flow from the S-7 Pump Station from theL-39 canal and S-10 culverts through STA-2 and thence the western portion of WCA-2A. This is much less than the 12-fold increase assumed by the District in carrying out its deterministic and probabilistic ecological risk assessments (Rumbold et al, 1999; Rumbold, 2000). These results are consistent with the earlier USEPA findings (Ambrose and Araujo, 1998) that the District's worst-case scenario likely substantially overestimated the post-ECP risks to wading birds foraging exclusively in the restored areas of the northern Everglades (Fink and Rawlik, 2000). This over-estimate provided the margin of safety required to offset the uncertainties in the risk calculations.

KEY FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

The analysis, integration, and synthesis of the general and Everglades-specific mercury literature and the relevant mercury monitoring, micrcocosm, mesocosm, and modeling results lead to the inevitable conclusion that the influences of surface water, sediment pore water, and sediment solids on net MeHg production and bioaccumulation are multi-dimensional, spatially heterogeneous, seasonally dynamic, and convolved. No one-variable or multi-variable empirical model can capture that complexity with the desired robustness, accuracy, precision, and confidence level for predicting the effect of post-ECP changes in water quantity and quality on post-ECP mercury risks to fish-eating wildlife. For the long term, the accurate prediction of post-restoration mercury risks to Everglades wildlife can only be accomplished by a mechanistic mathematical model that accurately represents the key physical, chemical, and biological processes governing mercury speciation, disposition, transport, transformation, and bioaccumulation and the physical, chemical, and biological factors that influence the routes and rates of those processes.

Recent modifications to the Everglades Mercury Cycling Model-II (E-MCM(II)) accommodate a number of the above discussed complexities, including the effect of phosphorus on Hg(II) and MeHg biodilution. The modified E-MCM (II) was used to predict the effect of a reduction of pre-BMP/pre-ECP phosphorus concentrations at the most impacted sites from about 175-180 ppb in 1994-1997 to 70 ppb in 1999-2002 and 10 ppb in 2006. At most, without a concomitant decrease in water flow, the loss of biodilution probably accounts for no more than a 250% increase in MeHg concentrations in top-predator fish, and no more than a 350% increase when the effects of halving flow and reducing surface water total phosphorus are combined.

Based on nearly seven years of intensive monitoring, research, and modeling, mercury scientists studying the Everglades have concluded that pore water sulfide is likely to be the best predictor of the MeHg production rate in the Everglades, and that the MeHg production rate, not biodilution, is likely to be the best predictor of MeHg bioaccumulation in the Everglades fish. Thus, here is no need to raise the proposed TP water quality standard of 10 ppb, exempt certain areas from its application, or delay its implementation based on earlier unrealistic estimates of increased mercury risks to fish-eating wildlife attributed to a loss of biodilution based on a one-variable empirical model developed with very limited data.

Ultimately, the solution to mercury pollution is not biodilution but source control. The focus of the efforts to understand and correct the Everglades mercury problem should now shift from empirical analysis of monitoring data to controlled laboratory and field studies of the underlying causes of the observed mercury effects. A number of such studies have been completed, are under way, or planned to start in the next fiscal year. The deeper mechanistic understanding of the effect of water, pore water, and sediment quality on the Everglades mercury cycle must then be translated in a realistic way into E-MCM(II), which will eventually be used to develop a mercury Total Maximum Daily Load (TMDL) for the Everglades and derivative emissions reductions.

REFERENCES

Ambrose, Jr., R.B. and R. Araujo. 1998. Applications of the Phase I Everglades Mercury Cycling Model. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Athens, GA, at the Third Annual Everglades Mercury Workshop, Sheraton West Palm Beach Hotel, West Palm Beach, FL. May.

Amirbahman, A., A.L. Reid, T.A. Haines, J.S. Kahl, and C. Arnold. 2002. Association of MeHg with dissolved humic acids. Environ. Sci. Technol. 36(4): 690-695

Amyot, M., D. Lean, and G. Mierle. 1997. Photochemical formation of volatile mercury in high arctic lakes. J. Environ. Toxicol. Chem. 16(10): 2054-2063.

Babiarz, C.L., J.P. Hurley, S.R. Hoffmann, A.W. Andren, M.M. Shafer, and D.E. Armstrong. 2001. Partitioning of THg and MeHg to the colloidal phase in freshwaters. Environ. Sci. Technol. 35(24): 4773-4782.

Balogh, S.J., Y. Huang, M.L. Meyer, and D.K. Johnson. 2002. Episodes of elevated MeHg concentrations in prairie streams. Environ. Sci. Technol. 36(8): 1665-1670.

Beijer, K., and A. Jernelov. 1979. Methylation of mercury in aquatic environments. In J. O. Nriagu (ed) The Biogeochemistry of mercury in the Environment. Elsevier/North Holland Biomedical Press, New York.

Benoit, J.M., C.C. Gilmour, R.P. Mason, and A. Heyes. 1999a. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Env. Sci. Technol. 33(6): 951-957.

Benoit, J.M., R.P. Mason and C.C. Gilmour. 1999b. Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and its implications for availability to methylating bacteria. J. Env. Toxicol. Chem. 8 (10): 2138-2141.

Benoit, J.M., C.C. Gilmour, and R.P. Mason. 2001. The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3). Environ. Sci. Technol. 35(1): 127-132.

Berman, M. and R. Bartha. 1986. Control of the methylation process in a mercury-polluted aquatic sediment. Environ. Pollution (Series B) 11: 41-53.

Berthouex, P.M., and L. C. Brown. 1994. Statistics for Environmental Engineers. Lewis Publishers. Boca Raton, FL

Bodaly, R.A., R.E. Hecky and R.J.P. Fudge. 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. Can. J. Fish. Aquat. Sci. 41: 682.

Branfireun, B.A., N.T. Roulet, C.A. Kelly, and J.W.M. Rudd. 1999. *In situ* sulphate stimulation of mercury methylation in a boreal peatland: toward a link between acid rain and MeHg contamination in remote environments. Global Biogeochem. Cycle 13: 743-750.

Brezonick, P.L. 1984. Trophic state indices: rationale for multivariate approaches in Lake and Reservoir Management, Proceedings 3rd Annual Conference of N. American Lake management Assoc., Knoxville, TN. EPA 440/5/84-001. USEPA. Washington, DC. May. pp 441-446.

Carlson, R. E. 1980. Using trophic state indices to examine the dynamics of eutrophication. Pages 218-21 in Restoration of Lakes and Inland Waters. Proc. Int. Symp. Inland Waters and Lake Restoration. Portland, Maine. EPA 440/5-81-010. USEPA, Washington, DC.

Chen, Y., J.-C. Bonzongo, W.B. Lyons, G.C. Miller. 1997. Inhibition of mercury methylation in anoxic freshwater sediment by group VI anions. J. Environ. Toxicol. Chem. 16(8): 1568-1574.

Choi, J. and J.W. Harvey. 2000. Quantifying time-varying ground-water discharge and recharge in wetlands of the northern Florida Everglades. Wetlands 20(3): 500-511.

Choi, M.H., J.J. Cech, Jr. and M.C Lagunas-Solar. 1998. Bioavailability of MeHg to Sacramento blackfish (orthodon microlepidotus): dissolved organic carbon effects. Environ. Technol. Chem. 17(4): 695-701.

Choi, S.-C., and R. Bartha, 1994. Environmental factors affecting mercury methylation in estuarine sediments. Bull. Environ. Contam. Toxicol. 53: 805-812.

Cleckner, L.B. P.J. Garrison, J.P. Hurley, M.L. Olson and D.P. Krabbenhoft. 1998. Trophic transfer of MeHg in the northern Florida Everglades. Biogeochemistry 40: 347-361.

Cleckner, L. B., C. C. Gilmour, J. P. Hurley, and D. P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. Limno. Oceanogr. 44(7): 1815-1825.

Compeau and Bartha. 1985. Sulfate-reducing bacteria. Principal methylators of mercury in anoxic estuarine sediments. Applied Environ. Microbiol. 50: 498-502.

Craig, P.J. and P.D. Bartlett. 1978. The role of hydrogen sulphide in environmental transport of mercury. Nature. 275: 635-637.

Dachs, J. S.J. Eisenreich, and R.M. Hoff. 2000. Influence of eutrophication on air-water exchange, vertical fluxes, and phytoplankton concentrations of persistent organic pollutants. Environ. Sci. Technol. 34(6): 1095-1102.

D'Itri, F.M., C.S. Annett and A.W. Fast. 1971. Comparison of mercury levels in an oligotrophic and eutrophic Lake. J. Mar. Technol. Soc. 5(6): 10-14.

Dmytriw, A. Mucci, M. Lucotte and P. Pichet. 1995. The partitioning of mercury in the solid components of dry and flooded forest soils and sediments from a hydroelectric reservoir, Quebec (Canada). Water, Air and Soil Pollution: 1099-1103.

Drake, H. 1994. Assessment of anaerobic microbial activities and potentials in "sediments" of Lake Okeechobee and Water Conservation Area 2A. Department of Microbial Ecology, University of Bayreuth, Bayreuth, Germany. Report to the South Florida Water Management District, West Plam Beach, FL. RFA No. KO 940057. July.

Driscoll, C.T., V. Blette, C. yan, C.L.Scofield, R. Munson, and J. Holsapple. 1995. Water, Air, and Soil Pollution 80: 499-508.

Dyrssen, D. and M. Wedborg. 1991. The sulphur-mercury(II) system in natural waters. Water Air Soil Poll. 56: 745-767.

Fink, L.E. 2000. ENR Project Mercury Studies: 1994-1999. Appendix 7-5. Everglades Consolidated Report. South Florida Water Management District, West Palm Beach, FL. January.

Fink, L.E. and P. Rawlik. 2000. Chapter 7: The Everglades Mercury Problem in the Everglades Consolidated Report, The South Florida Water Management District, West Palm Beach, FL. January.

Fink, L.E. 2001. The effect of surface and pore water quality on mercury bioaccumulation. Appendix 7-11 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, Fl.

Fink, L.E. 2002. The effect of effect of dryout and rewetting on mercury bioaccumulation. Appendix 2B-3 In The Everglades Consolidated Report 2002. South Florida Water Management District, West Palm Beach, Fl.

Frimmel, F.H., A. Innerz and H. Niedermann. 1984. Complexation capacities of humic substances isolated from fresh water with respect to copper(II), mercury (II) and iron (II,III). In: C.J.N. Kramer and J.C. Buinger, editors. Complexation of trace metals in natural waters. Matenus Nijhoss-Dr. W. Junk, The Hague: 329:343.

Garcia, E. and R. Carignan. 2000. Mercury concentrations in northern pike (*Esox lucius*) from boreal lakes with logged, burned, or undistrubed catchments. Can J. Fish. Aquatic Sci. 57 (Suppl.2): 129-135.

Gerrard, P.M. and V.L. St. Louis. 2001. The effects of experimental reservoir creation on the bioaccumulation of MeHg and reproductive success of tree swallows (Tachycineta bicolor). Environ. Sci. Technol. 35(7): 1329-1338.

Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. 71: 131.

Gilmour, C.C., E.A. Henry and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in sediments. Environ. Sci. Technol. 26(11): 2281-2287.

Gilmour, C.C., G.S. Riedel, J.D. Coates, and D. Lovley. 1996. Mercury methylation by Iron (III) reducing bacteria. Am. Soc. Of Microbiology 96th General Meeting, New Orleans, LA. May 19-23. Abstract (98) O-15: 356.

Gilmour, C.C., G.S. Ridel, M.C. Ederington, J.T. Bell, J.M. Benoit, G.A. Gill and M.C. Stordal. 1998a. MeHg concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry. 40: 327-345.

Gilmour, C.C., A. Heyes, J. Benoit, G. Reidel, J.T. Bell and G.Gill. 1998b. Distribution and biogeochemical control of mercury methylation in the Florida Everglades. Annual Report for 1998. Academy of Natural Sciences, Estuarine Research Center, St. Leonard, MD. Contract C-7690 with the South Florida Water Management District. June.

Gilmour, C.C., A. Heyes, J. Benoit, G. Reidel, J.T. Bell and G.Gill. 1999. Distribution and biogeochemical control of mercury methylation in the Florida Everglades. Final Report. Academy of Natural Sciences, Estuarine Research Center, St. Leonard, MD. Contract C-7690-A01 with the South Florida Water Management District. November

Henry, E.A. 1992. The role of sulfate-reducing bacteria in environmenatl mercury methylation in freshwater sediments. Ph.D. dissertaion. Harvard University. Cambridge, MA.

Grieb, T.M., C.T. Driscoll, S.P. Gloss, C.L. Schofield, G.L. Bowie, D.B. Porcella. 1990. Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Environ. Toxicol. Chem. 9: 919:930

Guentzel, J. 1997. The atmospheric sources, transport and deposition of mercury in Florida. Ph.D. Thesis, Florida State University, Tallahassee, Florida, USA. 177pp.

Guentzel, J.L., W.H. Landing, G.A. Gill, and C.D. Pollman. 2001. Processes influencing deposition of mercury in Florida. Environ. Sci. Technol. 35: 863-873.

Guentzel, J.L., R.T. Powell, W.M. Landing, and R.P. Mason. 1996. Mercury associated with collodial material in estuarine and open-coean environment. Marine Chemistry 55: 177-188.

Gun, J., A. Goifman, I. Shkrob, J. Kamyshny, B. Ginzburg, O. Hadas, I. Dor, A.D. Modestov, and O. lev. Formation of polysulfides in an oxygen rich freshwater lake and their role in the production of volatile sulfur compounds in aquatic systems. Environ. Sci. Technol. 34(22): 4741-4746.

Haitzer, M., G.R. Aiken, and J.N. Ryan. 2002. Binding of Mercury(II) to Dissolved Organic Matter: The Role of the Mercury-to-DOM Concentration Ratio. Environ. Sci. Technol. 36(16): 3564-3570

Hakanson, L. 1980. The quantitative impact of pH, bioproduction and Hg-contamination on the Hg- content of fish (pike). Environ. Pollut. (Series B) 1: 285-304.

Hintelmann, H., R. Ebinghaus, R-D Wilken. 1993. Accumulation of mercury(II) and MeHg by microbial biofilms. Water Res. 27(2): 237-242.

Howard, D. 1993. Trent Uiniversity. Petersborough, Ontario, Canada. Personal communication. 20 July.

Hurley, J.P., C.J. Watras, and N.S. Bloom. 1994. Distribution and flux of particulate mercury in four stratigied seepage lakes. In C.J. Watras and J.H. Huckabee (Eds) Mercury Polution: Integration and Synthesis. Lewis/CRC Press, Boca Raton, FL: 69-82.

Hurley, J.P., D.P. Krabbenhoft, L.B Cleckner, M.L. Olson, G. Aiken and P.J. Rawlik, Jr. 1998. System controls on aqueous mercury distribution in the northern Everglades, Biogeochemistry 40: 293-311.

Hurley, J.P., L.B. Cleckner, and P. Gorski. 1999. Everglades Nutrient Removal Project Mosquitofish Bioaccumulation Study. Draft Report. Prepared for the South Florida water Management District, West Palm Beach, FL. Contract (PC C-8691-0300). University of Wisconsin Water Chemistry Program, Madison, WI. May.

Jay, J.A., F.M.M. Morel, and H.F. Hemond. 2000. Mercury speciation in the presence of polysulfides. Environ. Sci. Technol. 34(11): 2196-2200.

Jensen, S. and A. Jernelov. 1969. Biological methylation of mercury in aquatic ecosystems. Nature. 223: 753-754.

Johnels, A.G., M. Olsson, and T. Westermark. 1967. Kvicksilver i fisk Var Foda 7: 67-103, as cited in Hakanson (1980).

Johnson, R. A. and D.W. Wichern. 1988. Applied Multivariate Statistical Analysis. Prentice-Hall. Englewood Cliffs, NJ.

Kelly, C.A., J.W.M Rudd, R.A. Bodaly, N.P. Roulet, V.L. St. Louis, A Heyes, T.R. Moore, S. Schiff, R. Aravena, K.J. Scott, B. Dyck, R. Harris, B. Warner, and G. Edwards. 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. Environ. Sci. Technol. 31(5): 1334-1344.

King, S.A. 2000. Mercury distribution, speciation, and transport in the Everglades Nutrient Removal treatment wetland. Ph.D. Thesis. Water Chemistry. University of Wisconsin. Madison, WI.

Koch, M. S., and P. S. Rawlik. 1993. Transpiration and stomatal conductance of two wetland macrophytes (Cladium jamaicense and Typha domingensis) in the subtropical Everglades. American Journal of Botany 80(10):1146-1154.

Krabbenhoft, D.P. and C.L. Babiarz. 1992. The role of groundwater transport in aquatic mercury cycling. Water Res. 28: 3119.

Krabbenhoft, D.P., J.P. Hurley, M.L. Olson and L.B. Cleckner. 1998. Diel variability of mercury phase and species distributions in the Florida Everglades. Biogeochemistry 40: 311-325.

Krabbenhoft, D.P., L.E. Fink, M.L. Olson, and P.S. Rawlik, II. 2000. The effect of dry down and natural fires on mercury methylation in the Florida Everglades. Conference Proceedings, International Conference on Heavy Metals in the Environment, University of Michigan, Ann Arbor. August.

Krabbenhoft, D.P., C.C. Gilmour, W.H. Orem, G. Aiken, M.L. Olson, J.F. DeWild, S.D. Olund, A. Heyes, G.S. Riedel, J.T. Vbell, H. Lerch, J.M. Benoit, and S. Newman. 2001. Interfacing

Process-Level Research and Ecosystem-Level Management Questions: Aquatic Cycling of Mercury in the Everglades (ACME) Phase II. Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Environments. Sheraton West Palm Beach, West Plam Beach, Florida. Sponsored by the U.S. Environmental Protection Agency, Washington, DC. May 8-10, 2001.

Krabbenhoft, D.P. and L.E. Fink. 2001. The effect of dry down and natural fires on mercury methylation in the Florida Everglades. Appendix 7-8 in Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL. January.

Lamers, L.P.M., H.B.M. Tomassen, and I. G.M. Roelofs. 1998. Sulfate induced eutrophication and phytotoxicity in freshwater wetlands. Environ. Sci. Technol. 32(2): 199-205.

Lange, T.R., D.A. Richard and H.E. Royals. 1998. Trophic relationships of mercury bioaccumulation in fish from the Florida Everglades. FINAL Annual Report. Florida Game and Fresh Water Fish Commission, Fisheries Research Laboratory, Eustis, FL. Prepared for the Florida Department of Environmental Protection, Tallahassee, FL. April.

Lange, T.R., D.A. Richard and H.E. Royals. 1999. Trophic relationships of mercury bioaccumulation in fish from the Florida Everglades. Annual Report. Florida Game and Fresh Water Fish Commission, Fisheries Research Laboratory, Eustis, FL. Prepared for the Florida Department of Environmental Protection, Tallahassee, FL. April.

Lindberg, S.E., H. Zhang and Meyers, T.P. 1999. Application of field methods and models to quantify mercury emissions from wetlands at the Everglades Nutrient Removal Project (ENR). Prepared by Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN and National Oceanic and Atmospheric Administration, ATDD, Oak Ridge, TN. Second Final Report. Everglades Mercury Air/Surface Exchange Study (E-MASE). Prepared for South Florida Water Management District, West Palm Beach, FL (C-6660). January.

Lindberg, S.E. and H. Zhang. 2000. Air/water exchange of mercury in the Everglades II: measuring and modeling evasion of mercury from surface waters in the Everglades Nutrient Removal Project. Science of Total Environ. 259: 135-143.

Lindberg, S.E., W. Dong, and T. Meyers. 2002. Transpiration of gaseous elemental mercury through vegetation in a subtropical wetland in Florida. Atmos. Environ. In Press.

Lockwood, R.A. and K.Y. Chen. 1974. Adsorption of Hg(II) by ferric hydroxide. Environ. Lett. 6(3): 151-166.

Loftus, W.F., J.C. Trexler, and R.D. Jones. 1998. Mercury transfer through an aquatic food web. Report submitted by Florida International University, Miami. Florida, to the Florida Department of Environmental Protection, Tallahassee, FL.

Mauro, J. B. N., and J. R. D. Guimaraes. 1999. Hg methylation potential in aquatic macrophytes of the Everglades Nutrient Removal Area. Report to the South Florida Water Management District, W. Palm Beach

Mauro, J. B. N., J. R. D. Guimaraes, and R. Melamed. 1999. Mercury methylation in macrophytes roots in a tropical lake. Water, Air, Soil Pollution 127: 271-280.

Marvin-DiPasquale, M. C. and D.G. Capone. 1998. Benthic sulfate reduction along the Chesapeake Bay central channel. I. Spatial trends and controls. Marine Ecology Progress series. 168: 213-228.

Marvin-DiPasquale, M.C. and R.S. Oremland. 1998. Bacterial MeHg degradation in Florida Everglades peat sediment. Environ. Sci. Technol. 32(17): 2556-2563.

Marvin-DiPasquale, M.M., J. Agee, R.S. Oremland, M. Thomas, D.P. Krabbenhoft, and C. G. Gilmour. 2000. MeHg degradation pathways: a comparison among three mercury-impacted ecosystems. Environ. Sci. Technol. 34: 4908-4916.

Marvin-DiPasquale, M.M., J. Agee and R.S. Oremland. 2001. Environmental controls on MeHg production and degradation by bacteria in Florida Everglades sediments. Draft report to the South Florida Water Management District under contract C-11719 by U.S. Geological Survey, Menlo Park, CA. April.

Mason, R. and A. Lawrence. 1999. Concentration, distribution, and bioavailability of mercury and MeHg in sediments of Baltimore Harbor, Maryland, USA. Environmental Toxicology and Chemistry 18(11): 2438-2447.

McCormick, P. V., P. S. Rawlik, K. Lurding, E. P. Smith and F. H. Sklar. 1996. Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. J. N. Am. Benthol. Soc. 15 (4): 433-449.

McCormick, P.V., S. Newman, G. Payne, S. Miao, and T.D. Fontaine. 2000. Chapter 3: Ecological Effects of Phosphorus Enrichment in the Everglades, Everglades Consolidated Report, South Florida water Management District, West Palm Beach, FL. January.

McMurtry, M.J., D.L. Wales, W.A. Scheider, G.L. Beggs and P.E. Dimond. 1989. Relationship of mercury concentrations in lake trout (Salvelinus namaycush) and smallmouth bass (Micropterus dolomieui) to the physical and chemical characteristics of Ontario lakes. Can. J. Fish Aqua. Sci. 46: 426-434.

Meili, M. 1994. Aqueous and biotic mercury concentrations in boreal lakes: model predictions and observations. In C.J. Watras and J.W. Huckabee (Eds.) Mercury Pollution – Intergation and Synthesis, Wiley Intersceince, NY: 99-105.

Mierle, G. and Ingram, R. 1991. The role of humic substances in the mobilization of mercury from watersheds. Wat. Air Soil Pollut., 56: 349 - 358.

Miles, C.J. and L.E. Fink. 1998. Monitoring and mass budget for mercury in the Everglades Nutrient Removal Project. Archives of Environ. Contam. and Toxicol. 35(4): 549-557.

Miles, C.J., H.A. Moye. E.J. Phlips, and B. Sargent. 2001. Partitioning of monoMeHg between freshwater algae and water. Environ. Sci. Tecnol. 35(21): 4277-4282.

Moye, H.A., C.J. Miles, E.J. Phips, B. Sargent, and K.K. Merritt. 2002. Kinetics and uptake mechansims for monomenthylmercry between freshwater algae and water. Environ. Sci. Technol. 36(16): 3550-3555

Miskimmin, B.M., J.W.M. Rudd, and C.A. Kelly. 1992. Influence of dissolved organic carbon, pH and microbial respiration rates on mercury methylation in lake water. Can. J. Fish. Aquatic Sci. 49: 17- 22.

Monod, J. 1942. As cited in Kiefer, D.A. and T. Enns. 1976. A steady-state model of carbon-, light-, and temperature-limited growth of phytoplankton in R. Canale, Editor. Modeling Biogeochemical Processes in Aquatoc Ecosystems. Ann Arbor Science, Ann Arbor, MI: 319-336.

Newman, S., H. Kumpf, J.A. Laing and W.C. Kennedy. 2001. Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54: 229-250.

Newman, S. and K. Pietro. 2001. Phopshorus storage and release in response to flooding: implications for Everglades stormwater treatment area. Ecological Engineering. In Press.

Norstrom, R. J., A.E. McKinnon and A.S.W. DeFreitas. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and MeHg residue levels in Ottawa River yellow perch (*Perca flavescens*). J. Fish Res. Board Can. 33: 248-267.

Olson, B.H. and R.C. Cooper. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. 10: 113-116.

Oremland, R.S., C.W. Culbertson, and M.R. Winfrey. 1991. MeHg decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. Appl. Environ. Microbiol. 57(1): 130-137.

Pak, K. and R. Bartha. 1998. Products of mercury demethylation by sulfidgens and methanogens. Bull. Environ. Contam. Toxicol. 61: 690-694.

Paterson, M.J., J.W.M. Rudd, and V. St. Louis. 1998. Increases in total and MeHg in zooplankton following flooding of a peatland reservoir. Env. Sci. Technol. 32(24): 3868-3874.

Pickhardt, P.C., C.L. Folt, C.Y. Chen, B. Klaue, and J.D. Blum. 2002. Algal blooms reduce the uptake of toxic MeHg in freshwater food webs. PNAS Biological Sciences: Ecology 99, 4419-4423.

Ravichandran, M., G.R. Aiken, M.M Reddy and J.N. Ryan. 1998. Enhanced dissolution of cinnabar (mercuric sulfide) by dissolved organic matter isolated from the Florida Everglades. Ennv. Sci. Technol. 32: 3205-3311.

Ravichandran, M. 1999. Interactions between mercury and dissolved organic matter in the Florida Everglades. Ph.D. Thesis. University of Colorado. Spring.

Rawlik, P. 2001a. Mercury concentrations in mosquitofish from treatment wetlands in the northern Everglades. Appendix 7-15 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, Fl.

Rawlik, P. 2001b. Stormwater Treatment Area 1 West: results of start-up mercury monitoring. Appendix 7-14 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, Fl.

Reddy, K. R., W. F. DeBusk, Y. Wang, R. DeLaune, M. Koch. 1991. Physico-Chemical Properties of Soils in the Water Conservation Area 2 of the Everglades. University of Florida, Gainesville.

Reddy, K.R., W. DeBusk, and Y. Wang. 1999a. Biogeochemical Processes Along a Nutrient Gradient. Data Report #3. Task 4.2. Rates of Microbial respiration under Field Conditions. Submitted to So. Fla. Water Management District, West Palm Beach, FL. Soil and Water Science Department, University of Florida, Gainesville, FL.

Reddy, K.R., W. DeBusk, and Y. Wang. 1999a. Biogeochemical Processes Along a Nutrient Gradient. Data Report #6. Task 6.1 Kinetics of Organic Phopshorus Mineralization. Submitted to So. Fla. Water Management District, West Palm Beach, FL. Soil and Water Science Department, University of Florida, Gainesville, FL.

Reddy, K.R., W. DeBusk, and Y. Wang. 1999a. Biogeochemical Processes Along a Nutrient Gradient. Data Report #9. Task 4.4 Rates of Decomposition of Dissolved Nutrient Concentrations as a Function of Soil Depth. Submitted to So. Fla. Water Management District, West Palm Beach, FL. Soil and Water Science Department, University of Florida, Gainesville, FL.

Reddy, M.M., A.R. Hoch, and G.R. Aiken. 1999. Calcite growth rate inhibition by aquatic humic substances. Proceedings of Advances in Crystal Growth Inhibition – American Chemical Society. August 22-26. New Orleans, LA.

Reddy, M.M., G. Aiken, P.F. Schuster. 1999. Hydroperiod-Driven Solute Transport at the Peat-Water Interface in the Florida Everglades: Hydrophobic Acid Diffusion from Peat. Unpublished Mansucript. U.S. Geological Survey, Boulder, CO. June.

Ribeyre, F. and A Boudou. 1994. Experimental study of inorganic and methylmercury bioaccumulation by four psecies of freshwater rooted macrophytes from water and sediment contamination sources. Ecotox. And Environ. Safety 28: 270-286.

Richardson, G.M, M. Egyed, and D. Currie. 1995. Does acid rain increase human exposure to mercury? A review and analysis of recent literature. J. Env. Toxicol. and Chem. 14(5): 809-813.

Riddle, S.G., H.H. Tran, J.G. Dewitt, and J.C. Andrew. 2002. Filed, laboratory, and X-ray absorption spectroscopic studies of mercury accumulation by water hyacinth. Environ. Sci. Technol. 36(9): 1965-1970.

Rodgers, D.W. 1994. You are what you eat and a little bit more: bioenergetics-based models of MeHg accumulation in fish revisited. In C.J. Watras and J.W. Huckabee, Mercury Pollution Integration and Synthesis, Lewis Publishers, Boca Raton: 427-439.

Rodgers, D.W., M. Dickman and X. Han. 1995. Stories from old reservoirs: sediment Hg and Hg methylation in Ontario hydroelectric developments. Water, Air and Soil Pollution 80: 829-839.

Rudd, J. W. M., and M. A. Turner. 1983. The English-Wabigoon River System: V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. 40: 2251-2259.

Rumbold, D.G. 2000. MeHg risk to Everglades wading birds: a probabilistic ecological risk assessment. Appendix 7.3b In Everglades Consolidated Report 2000. South Florida Water Management District, West Palm Beach, Fl.

Rumbold, D. and L. Fink. 1999. Appendix 7-4: Confounding Evidence for the "Inverse Relationship." I. Relationship between total phosphorus in the water column and mercury in mosquitofish. Everglades Interim Report, South Florida Water Management District, West Palm Beach, FL. January.

Rumbold, D.G., L. Fink, K. Laine, F. Matson, S. Niemczyk, and P. Rawlik. 2001a. Annual permit compliance monitoring report for mercury in Stormwater Treatment Areas and downstream receiving waters of the Everglades Protection Area. Appendix 7-9 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, Fl.

Rumbold, D.G., L. Fink, K. Laine, F. Matson, S. Niemczyk, and P. Rawlik. 2001b. Stormwater Treatment Area 6 follow-up mercury studies. Appendix 7-13 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, Fl.

SFWMD. 1995. Everglades Nutrient Removal Project: 1994 Monitoring Report. South Florida Water Management, Prepared for the Florida Department of Environmental Protection, Tallahassee, FL.

SFWMD. 1996. Everglades Nutrient Removal Project: 1995 Monitoring Report. South Florida Water Management, Prepared for the Florida Department of Environmental Protection, Tallahassee, FL.

SFWMD. 1997. Everglades Nutrient Removal Project 1996 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.

SFWMD. 1998. Everglades Nutrient Removal Project 1999 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.

SFWMD. 1999a. Everglades Nutrient Removal Project 1998 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.

SFWMD. 1999b. Final Report on the Effect of Best Management Practices on the Loading of Mercury Species to/from the Everglades Nutrient Removal Project: Monitoring Program (Project C-1). Submitted by the South Florida Water Management District, West Palm Beach, Florida, to the Floirda Department of Environmental Protection, Tallahassee, Florida, to fulfill the requirements of a Section 319 Grant (SP335/C-6663/4) from U.S. Environmental Protection Agency Region 4, Atlanta, GA.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, N.S. Bloom and R.J. Flett. 1994. The importance of wetlands as sources of MeHg to boreal forest ecosystems. Can. J. Fish. Aquatic Sci. 51: 1065-1076.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, R.J. Flett, and N.T. Roulet. 1996. Production and loss of MeHg and loss of THg from boreal forest catchments containing different types of wetlands. Environ. Sci. Technol. 30(9): 2719-2729.

Saouter, E., M. Gillman, R. Turner, and T. Barkay. Development and field validation of a microcosm to simulate the mercury cycle in a contaminated pond. Environ. Toxicol. Chem. 14(1): 69-77.

Scruton, D.A., E.L. Petticrew, L.J. LeDrew, M.R. Anderson, U.P. Williams, B.A. Bennett and E.L. Hill. 1994. MeHg levels in fish tissue from three reservoir systems in insular Newfoundland, Canada. In C.J. Watras and J.W. Huckabee, Mercury Pollution Integration and Synthesis, Lewis Publishers, Boca Raton: 441-455.

Sellers, P., C.A. Kelly, J.W.M. Rudd, A.R. MacHutcheon. 1996. Photodegradation of MeHg in lakes. Nature 380(25): 694-697.

Shin, E-B and P.A. Krenkel. Mercury uptake by fish and biomethylation mechanisms. J. Water Pollution Control Federation. 48(3): 1976

Sorenson, J.A., G.E. Glass, K.W. Schmidt, J.K. Huber, and G.R. Rapp, Jr. 1990. Airborne mercury deposition and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty northern Minnesota lakes. Environ. Sci. Technol. 24: 1716-1727.

Stumm, W. and J.J. Morgan. 1996. Aquatic Chemistry. 3rd Wilye Interscuience: 549-593.

Thibodeaux, L.J. 1996. Environmental Chemodynamics. 2nd Ed. John Wiley and Sons, Inc. pp 593.

Tsai, S-C, G. M. Boush, and F. Matsumura. 1975. Importance of Water pH in accumulation of inorganic mercury in fish. Bull. Environ. Contam. Toxicol. 13(2): 188-193.

USEPA. 1987. Quality Criteria for Water 1986. Office of Water Regulation and Standards. U.S. Environmental Protection Agency, Washington, DC. EPA 440/5-86-001. May.

Vaithiyanathan, P., C. J. Richardson, R, G. Kavanaugh, C. B. Craft and T. Barkay, 1996. Relationships of eutrophication to the distribution of mercury and the potential for MeHg production in the peat soils of the Everglades. Environ. Sci. Technol. 30(8): 2591-2597.

Vandal, G.M., R.P. Mason and W.F. Fitzgerald. 1991. Cycling of Volatile Mercury in Temperate Lakes. Water, Air and Soil Pollution 56:791-803.

Vandal, G.M., W.F. Fitzgerald, K.R. Rolfhus and C.H. Lamborg. 1995. Modeling the elemental mercury cycle in Pallette Lake, Wisconsin, USA. Water, Air and Soil Pollution 80: 529-538.

Wetzel, R.G. and Benfield, E.F. (1991) Extracellular interactions: Storage, redistribution, and interspecific communication. In: Chrost, R.J. (Ed) Microbial Enzymes in Aquatic Environments. Springer-Verlag, New York, USA: 6-28.

Wiener, J.G. 1986. Metal contamination of fish in low pH lakes and potential implications for piscivorous wildlife. Trans. 52nd N.A. Wildlife and Nat Res. Conf. 645657.

Wiener, J.G., W.F. Fitzgerald, C.J. Watras and R.G. Rada. 1990. Partitioning and bioavailability of mercury in an experimentally acidified Wisconsin lake. Environ. Toxicol. Chem. 9: 909-918.

Winfrey, M. R. and Rudd, J.W.M. 1990. Environmental factors affecting the formation of MeHg in low pH lakes. Environ. Toxicol. Chem. 9: 853.

Wolfe, M.F., D.M. Norman, R. Sulaiman. 1994. Mercury monitoring in wetlands birds and mammals at Clear Lake, CA. Toxicology Task Force, Seattle, WA.

Wood, J.M, F.S. Kennedy, and C.G. Rosen. 1968. Synthesis of MeHg compounds by extracts of methanogenic bacterium. Nature. 220: 173-174.

Xia, K, U.L. Skyllberg, W.F. Bleam, P.R. Bloom, E.A. Nater and P.A. Helmke. 1999. X-ray absorption spectroscopic evidence for the complexation of Hg(II) by reduced sulfur in soil humic substances. Env. Sci. and Technol. 33(5): 786-795.

Yin, Y. H.E. Allen, and C.P. Huang. Kinetics of mercury (II) adsoprtion and desorption on soil. Environ. Sci. Technol. 31(2): 496-503.

Zhang, Y-J., N.D. Bryan, F.R. Livens, and M.N. Jones. 1996. Complexing of Metal Ions by Humic Substances. Chapter 12 in Gaffney, J.S., N.A. Marley, and S.B. Clark: Humic and Fulvic Acids – Isolation, Structure, and Environmental Role. ACS Symposium Series 651: 194-206.

Zhang, H. and S.E. Lindberg. 2000. Air/water exchange of mercury in the Everglades I: the behavior of dissolved gaseous mercury in the Everglades Nutrient Removal Project. Science of Total Environ. 259: 135-143.

Attachment 1

UNIVARIATE LINEAR REGRESSION ANALYSIS RESULTS

[NOT SUPPLIED WITH REVIEW DRAFT]

Attachment 2

MULTIVARIATE LINEAR REGRESSION ANALYSIS RESULTS

[NOT SUPPLIED WITH REVIEW DRAFT]